ผลของไปเปอรีนต่อความบกพร่องด้านการเรียนรู้และความจำซึ่งเกิดจากภาวะสมองขาดเลือดชั่วคราวหรือสโคโปลามีนในหนูถีบจักร

นาย สุรชัย เพ็ญศิรินภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตร์มหาบัณฑิต
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EFFECTS OF PIPERINE ON LEARNING AND MEMORY IMPAIRMENT INDUCED BY TRANSIENT CEREBRAL ISCHEMIA OR SCOPOLAMINE IN MICE

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งานวิจัยนี้เป็นการศึกษาผลของไปเปอรีนซึ่งเป็นสารอัลคาลอยด์รสเผ็ดสำคัญในพริกไทยที่มีต่อความบกพร่องด้านความจำและภาวะเครียดออกซิเดชันในสมองอันเกิดจากภาวะสมองขาดเลือดหรือสโคโปลามีนในหนูถีบจักร โดยอาศัยตัวชี้วัดเป็นการทดสอบความจำสถานที่และการวัดไลปิดเพอร์ออกซิเดชันในสมอง ทั้งนี้สร้างภาวะสมองขาดเลือดชั่วคราวด้วยการผูกกั้นหลอดเลือดคอมมอนคาโรติดทั้งสองข้างเป็นเวลา 20 นาที แล้วประเมินความบกพร่องของการเรียนรู้และความจำสถานที่เป็นเวลา 5 วันติดต่อกันด้วยสระน้ำกลของมอริส ปรากฏว่าหนูซึ่งผูกกั้นหลอดเลือดใช้เวลาในการว่ายน้ำค้นหาแท่นพักซึ่งซ่อนอยู่ยาวนานขึ้นเมื่อเทียบกับหนูซึ่งได้รับเพียงการผ่าตัด การฉีดไปเปอรีนเข้าช่องท้องในขนาด 0.1 และ 0.5 มก/กก/วัน เป็นเวลา 5 วัน หลังจากการผูกกั้นหลอดเลือด บรรเทาความบกพร่องด้านความจำนี้ได้อย่างชัดเจน ขณะที่การฉีดไปเปอรีนในลักษณะเดียวกันด้วยขนาดที่สูงกว่า (1 และ 5 มก/กก/วัน) แสดงผลป้องกันความจำเสื่อมได้น้อยกว่า ยังพบผลดีของไปเปอรีนในการทดสอบความจำสถานที่กับหนูปกติและหนูซึ่งได้รับเพียงการผ่าตัด อย่างไรก็ตามผลลัพธ์ที่ได้ค่อนข้างต่ำกว่าการทดสอบกับหนูซึ่งผูกกั้นหลอดเลือด นอกจากนั้นการฉีดไปเปอรีนติดต่อกัน 5 วัน ในทุกขนาดทดสอบ ไม่แสดงผลใดๆ ที่เด่นชัดต่อสมรรถนะการเคลื่อนที่ของหนูปกติ

ในทางตรงกันข้าม การฉีดไปเปอรีนเข้าช่องท้องติดต่อกัน 5 วัน ในขนาด 0.1, 0.5, 1 และ 5 มก/กก/วัน ไม่แสดงผลบรรเทาความบกพร่องด้านความจำสถานที่อันเกิดจากการฉีดสโคโปลามีนในหนูถีบจักร ผลทดลองดังกล่าวจึงชี้แนะว่าไปเปอรีนอาจไม่มีฤทธิ์กระตุ้นหรืออันตรกริยากับระบบโคลิเนอร์จิคในระบบประสาทส่วนกลาง

ปริมาณไลปิดเพอร์ออกซิเดชันในสมองที่วัดเมื่อวันที่ 5 หลังการผ่าตัด (วัดโดยการวิเคราะห์ TBARs) ของหนูซึ่งผูกกั้นหลอดเลือดมีระดับสูงกว่าเมื่อเปรียบเทียบกับหนูซึ่งได้รับเพียงการผ่าตัด ระดับ TBARs ที่สูงขึ้นนี้จะลดลงอย่างชัดเจนเมื่อฉีดไปเปอรีนเข้าช่องท้องในขนาด 0.1 และ 0.5 มก/กก/วัน เป็นเวลา 5 วันขณะที่การฉีดไปเปอรีนในลักษณะเดียวกันด้วยขนาดที่สูงขึ้น (1 และ 5 มก/กก/วัน) สามารถลดระดับ TBARs ดังกล่าวลงได้บ้าง นอกจากนั้นยังสังเกตุเห็นผลดีของไปเปอรีนต่อระดับไลปิดเพอร์ออกซิเดชันในสมองได้พอควรในหนูซึ่งได้รับเพียงการผ่าตัด

โดยสรุปผลการทดลองเหล่านี้ชี้แนะว่าการใช้ไปเปอรีนมีผลดีต่อภาวะบกพร่องด้านความจำและการเพิ่มขึ้นของไลปิดเพอร์ออกซิเดชันในสมองอันเกิดจากการผูกกั้นหลอดเลือดในหนูถีบจักร และจากความสัมพันธ์อย่างใกล้ชิดระหว่างผลของไปเปอรีนต่อตัวชี้วัดภาวะสมองบาดเจ็บทั้งสองย่อมอนุมานได้ว่าผลบรรเทาภาวะบกพร่องด้านความจำอันเกิดจากการผูกกั้นหลอดเลือดอาจเกี่ยวข้องอย่างน้อยบางส่วนกับคุณสมบัติต้านออกซิเดชันของไปเปอรีน ดังนั้นจึงเป็นการสมควรจะพิจารณานำไปเปอรีนไปศึกษาต่อเพื่อพัฒนาใช้เป็นยาช่วยที่เป็นไปได้ในการรักษาความผิดปกติเกี่ยวกับความเสื่อมของระบบประสาท

ภาควิชา เภสัชวิทยา ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชวิทยา ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2545

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KEYWORDS: PIPERINE, CEREBRAL ISCHEMIA, MEMORY IMPAIRMENT, LIPID PEROXIDATION, SCOPOLAMINE

SURACHAI PENSIRINAPA: EFFECTS OF PIPERINE ON LEARNING AND MEMORY IMPAIRMENT INDUCED BY TRANSIENT CEREBRAL ISCHEMIA OR SCOPOLAMINE IN MICE. THESIS ADVISOR: ASST. PROF. SURACHAI UNCHERN, Ph.D. 89 pp. ISBN 974-17-2958-8.

Effects of piperine, a major pungent alkaloid in pepper, on the cognitive deficit and cerebral oxidative stress induced by cerebral ischemia or scopolamine were studied in mice by using spatial memory task and measurement of lipid peroxidation in the brain. Transient cerebral ischemia was induced by 20-min bilateral common carotid artery occlusion (2VO) and the impairment of spatial learning and memory was subsequently evaluated for 5 consecutive days by a Morris water maze. The 2VO-mice displayed a delay in swimming time to find the hidden platform (escape latency) when compared to sham-operated mice. The 5-day intraperitoneal (i.p.) administration of piperine, at 0.1 and 0.5 mg/kg/day after the 2VO, markedly attenuated this cognitive deficit while the same administration at higher doses (1 and 5 mg/kg/day) showed lesser preventive effect on the deficit. Beneficial effects of piperine on spatial memory task were also found in normal and sham-operated mice. However, the magnitude of effects was relatively small comparing to that observed in 2VO mice. In addition, 5-day piperine administration at all test doses did not show any significant effects on locomotor activity of normal mice.

On the other hand, the 5-day i.p. administration of piperine at 0.1, 0.5, 1, and 5 mg/mg/kg/day did not attenuate spatial memory impairment induced by scopolamine administration in mice. This result suggested that piperine might have no cholinomimetic activity or cholinergic interactions in the CNS.

The brain lipid peroxidation (as measured by TBARs assay) of 2VO-mice at 5 days after the occlusion was significantly increased when compared to sham-operated mice. This increase was markedly attenuated by 5-day i.p. administration of piperine at 0.1 and 0.5 mg/kg/day while the same administration at higher doses (1 and 5 md/kg/day) showed modest attenuation on the increase. Moderate beneficial effects of piperine on brain lipid peroxidation were also noticed in sham-operated mice.

Taken together, these results suggested that piperine administration had beneficial effects on 2VO-induced cognitive deficit and brain lipid peroxidation increase in mice. The close correlation between effects of piperine on both indications of brain injury also implied that the attenuation of 2VO-induced cognitive deficit may involve, at least partly, the antioxidant property of piperine. Conceivably, piperine may be considerable for further study as a possible adjunctive medication in the treatment of neurodegenerative disorders.

Department: Pharmacology Student's signature.....

Field of study: Pharmacology Advisor's signature.....

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ABBREVIATIONS

2VO	=	two vessel occlusion
AD	=	Alzheimer's disease
CNS	=	Central nervous system
DMSO	=	Dimethyl sulfoxide
MDA	=	Malondialdehyde
MWM	=	Morris water maze
NE	=	North east
NW	=	North west
SE	=	South east
SW	=	South west
NSS	=	Normal saline solution
P	=	Piperine
PNS	=	Peripheral nervous system
Scop	=	Scopolamine
SDS	=	Sodium dodecyl sulfate
TBARs	=	2-Thiobarbituric acid reactive substance

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Chapter 1

General Review of the Literature

Introduction

Piperine is the most common alkaloid in the *Piper* species of Piperaceae family which is one of the oldest and the most important member of the spices. The two forms of the spice, black pepper and white pepper, are obtained from the fruits and seeds of *Piper nigrum*, black pepper consisting of the dried ground fruits and white pepper consisting of the dried ground seeds. They are used extensively as a condiment and flavoring for all types of savory dishes, for preserving and pickling, and in the manufacture of sauces, ketchups, and brandy (Govindarajan, 1977). The pungency of pepper is due to the presence in the fruit of various resins and a yellow crystalline alkaloid, piperine, which is present to the extent of 4.5-8%. Piperine is the *trans-trans* isomer of 1-piperoylpiperidine and contains the methylenedioxy moiety. It has the composition of $C_{17}H_{19}O_3N$ and molecular weight of 285.16 (Atal et al., 1975). Physicochemically, piperine is a neutral or slightly alkaline crystalline substance, insoluble in water but readily soluble in alcohol and when pure is colorless, and without taste or smell.

Figure 1 Fruits and seeds of *Piper nigrum* Lam.

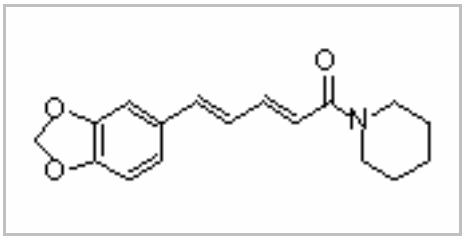

Figure 2 : Chemical structure of piperine

Traditional Uses of Pepper

Uses of pepper have been acclaimed in traditional medicine since the ancient time. It is widely used as an aromatic carminative stimulant. Externally applied, pepper is regarded as a useful remedy in hemorrhoid, and in relaxed conditions of the rectum attended with prolapsus, or when locally applied as a gargle. Pepper has been found useful in relaxed uvula, paralysis of tongue, and in other affections of the mouth or throat. In addition, pepper is also employed in folklore medicine for the treatment of epilepsy, asthma, bronchitis, pyrexia and abdominal disorders.

Biological Activities of Piperine

Piperine is considered to be the active principle of various *Piper* species which are employed in folklore medicine. Several studies on the biological activity of piperine indicated a wide variety of effect on several systems such as the central nervous system (CNS), cardiovascular system, respiratory system, and hepatic drug metabolism (Atal et al., 1985).

Pharmacological Effects of Piperine on the Central Nervous System

Piperine was shown to possess CNS depressant property (Woo et al., 1979; Pei, 1983; Pei et al., 1980). Pharmacological studies indicated that piperine and several of its derivatives protect rats and mice against various kinds of experimental convulsions, including those induced by maximal electroshock, picrotoxin and strychnine (Pei, 1979, 1983; Woo et al., 1979). They also showed

sedative-hypnotic, tranquilizing and muscle-relaxing actions and can intensify the depressive action of other depressants, when used in combination. Antiepilepsirine, one of the derivatives of piperine, was used as an antiepileptic drug in treating different types of epilepsy. It had been proved effective and was widely used in China (Pei, 1983). It appeared that piperine and its derivatives affect the central serotonergic system and this action might be related to the anticonvulsant property of piperine (Liu et al., 1984; Mori et al., 1985). Recent research suggested that, piperine was significantly blocked convulsions induced by intracerebroventricular injection of kainate but have no or exert only slight effects on convulsions induced by L-glutamate and N-methyl-D-aspartate (NMDA). Although piperine did block convulsions induced by kainate, the compound does not appear to act as a kainate receptor antagonist (D'Hooge et al., 1996). Although it was well demonstrated that piperine has anticonvulsant property, some studies reported that piperine, especially at high doses, has respiratory stimulant and convulsant properties in various laboratory animals (Kulshrestha et al., 1969; Singh et al., 1973).

Other Pharmacological Activities of Piperine

Beside its remarkable effects on the CNS, piperine showed a wide variety of pharmacological activities in experimental animals, such as antipyretic and analgesic activities (Lee et al., 1984); anti-inflammatory activity (Lee et al., 1984; Mujumdar et al., 1990a; Dhuley et al., 1993); antiamoebic activity (Ghoshal et al., 1996); antifertility activity (Piyachaturawat et al., 1982, 1991).

Many spice principles contain antioxidant or free-radical scavenging activities. However, many studies indicated that piperine contains weak antioxidant and free-radical scavenging activities comparing to other spice principles such as curcumin from turmeric, capsaicin from red chillies, and eugenol from cloves (Krishnakantha and Lokesh, 1993; Reddy and Lokesh, 1992; Joe and Lokesh, 1994). However, piperine was reported in one study to exert a weak hepatoprotective activity in mice (Koul and Kapil, 1993).

Cerebral Ischemia-Induced Brain Injuries

After ischemia, tissue injury is a main cause of disorders found in brain, heart, liver and kidney. Although pathological mechanisms underlying ischemic injury are not well-understood, they are supposed to involve hypoxia, free radical damage, and inflammatory responses (Haba et al., 1991; Paller, 1994). The role of oxygen free radicals in ischemic injury has received a great deal of attention and it has been found that oxygen free radical-mediated lipid peroxidation is, therefore, one of the most important reactions in the progression of ischemic injury (De Vecchi et al., 1998; Haba et al., 1991). It is important to clarify the role of oxidative stress in ischemia and to develop a new drug for prevention of the injury.

Ischemic injury in the brain induced by stroke, cardiac arrest, and brain injury causes neuronal death and dementia. Recent evidence suggests that the cellular damage induced by cerebral ischemia as well as bilateral common carotid artery occlusion is at least partly due to oxidative damage caused by free radicals and lipid peroxidation (Haba et al., 1991; Kogure et al., 1985). Characteristics of cerebral ischemia models are based on similarities with syndromes of human cerebrovascular disease (Molinari, 1976) and, above all, the focal ischemia models are the most pertinent to stroke in humans (Garcia, 1984).

Generation of free radicals is closely related to ischemic injuries. It has long been suggested that oxygen free radicals contributed to ischemia brain damage (Siesjo, 1992). Although many investigators have attempted to clarify the pathophysiological role of free radicals in cerebral ischemia, it has not been fully explained by the *in vivo* cerebral ischemia models. However, inhibition of free radical formation or lipid peroxidation prevents the progression of neuronal damage (Haba et al., 1991; Kogure et al., 1985). Antioxidants such as α-tocopherol (vitamin E) and indomethacin have neuroprotective effects on the cell after ischemia (Haba et al., 1991; Ito et al., 1994; Kogure et al., 1985). Although several reports suggest that antioxidant has a protective effect on the toxicity of amyloid β (A_β) in cell cultures (Goodman et al., 1994; Rothman et al., 1993), little is known about the effect of antioxidants on behavioral deficits and histological injury caused by ischemia *in vivo*.

Scopolamine-Induced Amnesia

Degeneration of basal forebrain cortical cholinergic neurons occurs in the brains of subjects with Alzheimer's dementia, and this correlates well with the degree of cognitive impairment (Bowen et al., 1976; Whitehouse et al., 1982). It is well known that cholinergic neuronal systems play an important role in the cognitive deficits associated with aging and neurodegenerative diseases (Bartus et al., 1982; Newhouse, 1990).

A number of cholinesterase inhibitor has been shown to improve cognitive function in dementia subjects (Stern et al., 1987; Knapp et al., 1994; Canal and Imbimbo, 1996; Rogers et al., 1998). On the other hand, anticholinergic drugs, like scopolamine, can disrupt short-term or working memory in humans and animals. (Stevens, 1981; Beatty et al., 1998; Kopelman and Corn, 1988). It was shown that cholinesterase inhibitors, including physostigmine, tacrine, donepezil, and heptylphysostigmine, antagonize the effect of scopolamine on spatial memory in the Morris water maze and passive avoidance (Dawson et al., 1991; Yoshida and Suzuki, 1993). Moreover, in Alzheimer patients, the drugs produced a dose-related effect on cognitive function that was correlated with the degree of acetylcholinesterase inhibition in the cerebrospinal fluid. (Cutler et al., 1998).

Rationale of the Study

Pepper has been consumed worldwide without reported toxicity. In addition, pepper and piperine have been widely used in folklore medicine to treat a variety of central nervous system disorders, especially epilepsy. Therefore, it is conceivable that they may be therapeutically useful in other neurological deficits, such as dementia (Stevens, 1981; White house, 1982). In addition, piperine has antioxidant property which may suggest its potential to alleviate oxidative stress-induced neurodegenerative process.

According to folklore medicine in some Asian countries, daily intake of pepper in the elderly has been claimed to reduce the impairment of learning and memory. However, there has been no report on the memory-enhancing or memory impairment-attenuating effects of pepper or piperine. Therefore, it is conceivable to

investigate the beneficial effect of piperine, the major alkaloid in pepper, on memory impairment in animals models which may shed some light on the hope for an alternative pharmacotherapy of dementia and neurodegenerative disorders.

Hypothesis

1. Piperine may attenuate learning and memory impairment induced by experimental manipulations (transient cerebral ischemia and scopolamine administration).

2. Beneficial effects of piperine on learning and memory may be, at least in part, due to its antioxidant activity.

Objectives of the study

1. to assess the effect of piperine treatment on memory impairment in mice induced by two procedures; bilateral common carotid artery occlusion and scopolamine administration, in Morris water maze test.

2. to investigate the possible mechanisms underlying beneficial effects of piperine on memory impairment, in particular, related to cerebral ischemia-induced oxidative damages in the brain by using lipid peroxidation as an indicator.

Chapter 2
Materials and Methods

1. Animals

All experiments in this study were performed by using male Swiss albino mice, eight-week old, and weighing 30-35 g. All animals were obtained from the National Animal Center, Mahidol University, Nakornpathom. Prior to testing, they were housed 8 mice per cage for one week in the Animal House of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, at 25 ± 2 °C and a 12 hr diurnal dark/light cycle with food pellets and water *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.

2. Meterials

Piperine [Sigma]
Scopolamine hydrobromide [Sigma]
Dimethysulfoxide (DMSO) [BDH]
Butylated hydroxytoluene [Sigma]
Phosphotungstic acid [Sigma]
Sodium dodecyl sulfate [Sigma]
2-Thiobarbituric acid [Sigma]
Malondialdehyde (MDA) [Sigma]

3. *In vivo* Measurement: Behavioral Analysis

3.1 Morris water maze : Spatial memory test

The procedure used was a modification of that described by Morris (1984). The Morris water maze consisted of a circular pool, painting with black color, which was 150 cm in diameter and 45 cm in height. It was filled to the depth of 30 cm with water at a temperature of 25 ± 2 °C. The pool was divided into four

quadrants of equal area NE, NW, SE, SW. A hidden platform (escape platform) made of clear plexiglass, 10 cm in diameter, was placed 37.5 cm from the wall in the middle of one quadrants. The platform was submerged 1 cm below the surface of water.

The mouse was introduced into the pool in the SE or NE quadrant. The point of entry into the pool was changed every time, the same observer measured the time taken for the mouse to find the escape platform (escape latency). During a particular trial, the mouse was able to escape from the water only by climbing onto submerged platform. For each mouse, The location of the hidden platform remained unchanged throughout the experiment. A trial was terminated as soon as the mouse found the platform; if it failed to do so within 120 s, it was placed on the platform by the experimenter's hand. The animal was allowed to stay on the platform for 30 s and then removed from the pool. The mouse was given five trials per day for 5 consecutive days with an inter-trial interval of approximately 20 min. The decrease in day-to-day escape latency during the experiment indicated the improvement in spatial learning and memory.

3.2 Criteria for the selection of mice

On day 1, the mouse was introduced into the pool for water adaptation and pretraining before the study. Only mice with normal swimming profile were used in the following steps. The mouse's swimming path should be in random directions, without circular pattern around the pool, and it could find the hidden platform within 120 seconds. The selected mice were then subjected to bilateral common carotid artery occlusion procedure.

3.3 Piperine pretreatment

To study the effect of piperine on spatial learning and memory of normal mice, seven groups of mice were used (8 mice per group). One group of animals was injected intraperitoneally not exceed 0.1 ml DMSO and served as the control group. Other six groups of animals were injected intraperitoneally with piperine solutions (in DMSO) at various doses (0.1, 0.5, 1, 5, 10, 15 mg/kg). Thirty minutes after the injection, all mice were subjected to Morris water maze test for spatial

learning and memory task. In most experiments, piperine administration was given for 5 consecutive days (subacute treatment)

3.4 Transient cerebral ischemia-induced learning and memory impairment.

Transient cerebral ischemia was induced in mice by the method of Pulsinelli and Brierley (1979) using two-vessel-occlusion (2VO) procedure. Mice were anesthetized with sodium pentobarbital (Nembutal sodium solution, 60 mg/kg, intraperitoneal injection). Under deep anesthesia, the neck skin of mice was vertically incised and their common carotid arteries were exposed by pulling with threads. Then the bilateral common carotid arteries were occluded for 20 minutes using microvascular clamps. Body temperature during the ischemia was maintained by using heating lamp. Subsequently, both clamps were removed and both arteries were inspected for immediate reperfusion. At the same time, the control (sham-operated) mice had their bilateral common carotid arteries exposed, but did not have their carotid arteries occluded. At 24 hours after transient global cerebral ischemia, 2VO mice were tested with Morris water maze to analyze deficits in spatial learning and memory.

To study the effect of piperine on spatial learning and memory impairment of mice induced by cerebral ischemia, ten groups of animals were used: one group of sham-operated animals (n = 8); one group of 2VO animals (n = 8); four groups of sham-operated and four groups of 2VO mice (n = 8 per group) receiving intraperitoneal injections of test substance (0.1, 0.5, 1, and 5 mg/kg of piperine, respectively). Piperine was administered 30 minutes before testing for spatial learning and memory in Morris water maze.

3.5 Scopolamine-induced learning and memory impairment.

To study the effect of piperine on spatial learning and memory impairment of mice induced by scopolamine, groups of mice (8 mice per group) were daily injected intraperitoneally with DMSO (0.1 ml : as control), or piperine (0.1, 0.5, 1, and 5 mg/kg), followed 10 min later by scopolamine (0.5, 1 mg/kg) or 0.1 ml NSS.

All mice were tested for spatial learning and memory 20 min after the injection of scopolamine in the same manner as described for 2VO model (Bcatty, 1986).

3.6 Locomotor activity measurement

The activity cage consists of plexiglass chamber and counting unit was used. 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 read at 5 min intervals.

Motor activity was measured in the activity cage for a total period of 105 min. A basal locomotor activity of each mouse was established by allowing a control period of 45 min before removing it for the administration of test substance (piperine 0.1, 0.5, 1, and 5 mg/kg IP), DMSO, or NSS (as control). Immediately after the administration of piperine, DMSO, or NSS, the animals were returned to their activity cages and locomotor activity was further monitored for 60 min.

The experiment was carried out between 7.30-11.00 a.m. Mice were divided into 6 groups of 8 animals each. Two groups were used as control groups (DMSO and NSS). The other 4 groups were used as the treatment groups.

4. *In vitro* Measurement: Lipid Peroxidation Assay

Piperine-induced oxidative stress was evaluated by measuring mouse brain-derived TBARS, an indicator of lipid peroxidation (Agar et al., 1999, Adonaylo, 1995).

After 24 hr of transient global cerebral ischemia, mice were daily treated with piperine in various doses (0.1, 0.5, 1 and 5 mg/kg, IP) for 5 days. Thirty minutes after the last dose, the animals were killed by cervical dislocation and the whole brain was quickly removed. The cerebral cortex was subsequently dissected out, rinsed with iced-cold saline and weighed. The tissue homogenates (1 g/10 ml) were prepared in ice-cold 20 mM Tris-HCl, 0.14 M NaCl buffer (pH 7.4). Half milliliter of tissue homogenate was mixed with 0.1 ml of 4% (w/v) butylated hydroxytoluene in ethanol. Then samples were mixed with 0.5 ml of 3 % (w/v) sodium dodecyl sulfate, 2 ml of 0.1 M HCl, 0.3 ml of 10 % (w/v) phosphotungstic acid, and 1 ml of 0.7 % (w/v) 2-thiobarbituric acid. After vortexing, samples were

incubated for 45 min in boiling water, then cool in tap water and TBARs were extracted with 3 ml of n-butanol. Following centrifugation at 1000 × g for 10 min, optical density of the supernatant was measured by a spectrophotometer at 555 nm.

TBARs values are expressed as malondialdehyde (MDA) equivalents. The MDA, prepared from 1, 1, 3, 3,- tetraethoxypropane, was used as the standard.

5. Statistical 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 Tukey HSD 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 3
Results

Part I : Effects of piperine on spatial learning and memory performance in normal mice.

Various doses of piperine were tested with normal mice to determine the effective dose range for the following experiments on mice with learning and memory impairment.

The profile of daily escape latency times of mice treated with piperine at different doses, DMSO and NSS, are summarized in Figures 3 and 4. Preliminary experiments revealed that administration of DMSO suppressed spatial memory performance of normal mice as compared to that of normal mice treated with NSS.

After treatment with piperine at doses of 0.1, 0.5, 1, 5, 10, and 15 mg/kg, it appeared that piperine clearly facilitated spatial memory performance at doses of 0.1 and 05 mg/kg when compared to the DMSO-treated as well as the NSS-treated groups. However piperine at doses of 1 and 5 mg/kg had no significant effects on spatial memory performance as compared with the DMSO-treated group. Furthermore, treatment of mice with high doses of piperine (10 and 15 mg/kg) significantly suppressed the spatial memory performance of normal mice as compared to DMSO-treated group. Piperine at high doses might exert some toxic effects to mice thereby increasing swimming time to find the hidden platform. Therefore, all subsequent experiments on mice with memory impairment were done with piperine at non-toxic dose range of 0.1 to 5 mg/kg.

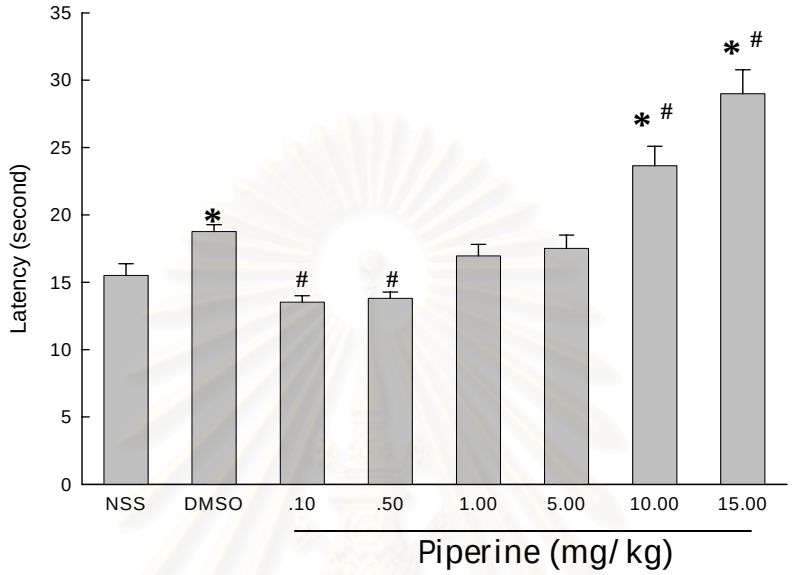

Figure 3 : Effects of pretreatment with different doses of piperine (0.1, 0.5, 1, 5, 10, and 15 mg/kg, i.p.) on performance of normal mice in the Morris water maze. Values are expressed as the mean ± SEM (n=8) of average escape latency times from a 5-day spatial memory task. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant.

* Significantly different from NSS-treated group.

Significantly different from DMSO-treated group.

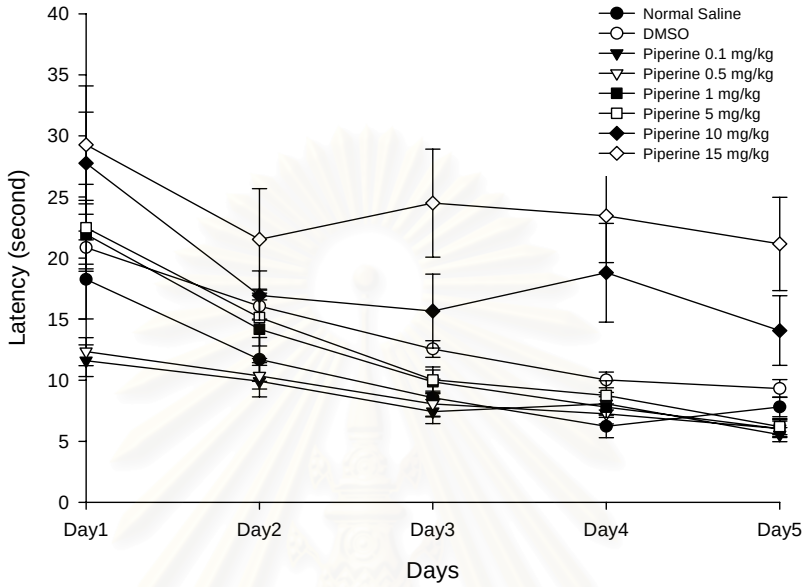

Figure 4 : Effects of pretreatment with different doses of piperine (0.1, 0.5, 1, 5, and 10 mg/kg, i.p.) on performance of mice in the Morris water maze. Values are expressed as the mean ± SEM (n=8) of daily escape latency times during 5 consecutive days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant.

Part II : Effects of transient cerebral ischemia on spatial learning and memory performance in mice.

The water maze performance in bilateral common carotid artery occluded (2VO) and Sham-operated animals as measured by latency to reach the submerged platform during 8 experimental days was summarized in Figure 5. There was no significant difference in escape latency between Sham-operated (as the control) and 2VO groups before the treatment (day 0). After transient cerebral ischemia, the escape latency in 2VO mice was delayed as compared to Sham-operated mice. However, significant differences were found only during days 1-5 but not during days 6-8. Therefore, all further experiments on the spatial memory performance of mice were done with a training schedule of 5 consecutive days.

The comparison of spatial memory performance among normal saline control, Sham-operated control, and 2VO-operated groups was shown in Figure 6. The mean search times to find the hidden platform in the pretraining day (day 0) did not differ among these three groups. However, during days 1-5, there were significant delayed escape latency times in 2VO- and Sham-operated mice as compared to normal mice. As brain damage caused by cerebral ischemia of 2VO in mice is larger than that Sham-operated group and the naive normal saline group. In addition to, from the data obtained, it was found that, the Sham-operated group showed the mean escape latency longer than the naive normal saline group. Also, the increased mean escape latency in Sham-operated group showed that it induced the brain injury compared to the naive normal saline group.

Part III : Effects of piperine on spatial learning and memory performance in mice with transient cerebral ischemia.

A. Effects of treatment with DMSO on spatial learning and memory performance in mice with transient cerebral ischemia.

DMSO (a solvent for piperine) clearly exerted no effect on spatial learning and memory performance in Sham- and 2VO-operated mice as shown in Figure 7.

B. Effects of treatment with piperine on spatial learning and memory performance in mice with transient cerebral ischemia.

Effects of treatment with piperine at doses of 0.1, 0.5, 1 and 5 mg/kg on spatial learning and memory performance in mice with bilateral common carotid artery occlusion were shown in Figures 8, 9, 10, 11, 12, 13 and 20. Piperine administration markedly attenuated the memory deficits found in 2VO mice. They were faster in finding the hidden platform (shortened escape latency) than the DMSO-treated (control) mice throughout the training period.

C. Effects of treatment with piperine on spatial learning and memory performance in Sham-operated mice.

Effects of treatment with piperine at doses of 0.1, 0.5, 1 and 5 mg/kg compared to DMSO treatment in Sham-operated mice were shown in Figures 14, 15, 16, 17, 18, 19 and 20. Piperine administration also attenuated the memory deficits found in Sham-operated mice. They were significantly faster in finding the hidden platform (shortened escape latency) than the DMSO-treated (control) mice. However, the magnitude of effects in Sham-operated mice were less than that in 2VO mice. Only piperine at doses of 0.1 and 0.5 mg/kg significantly improved learning and memory performance whereas doses of 1 and 5 mg/kg did not show any beneficial effects.

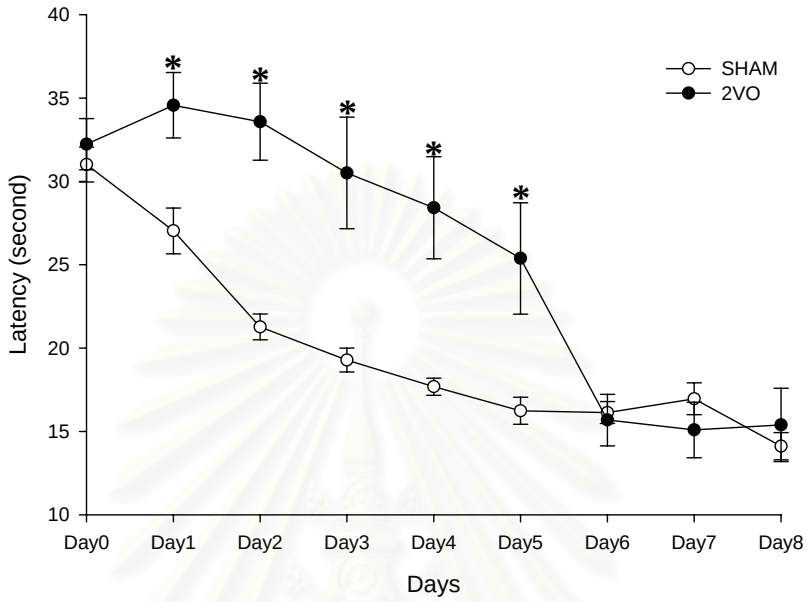

Figure 5 : Effects of 2VO and Sham-operation on Morris water maze performance in mice. The escape latency values are expressed as the mean ± SEM (n=8) during 8-day training schedule. A significance level of $P < 0.05$ was considered as a significant difference.

*Significantly different from value in Sham-operated group.

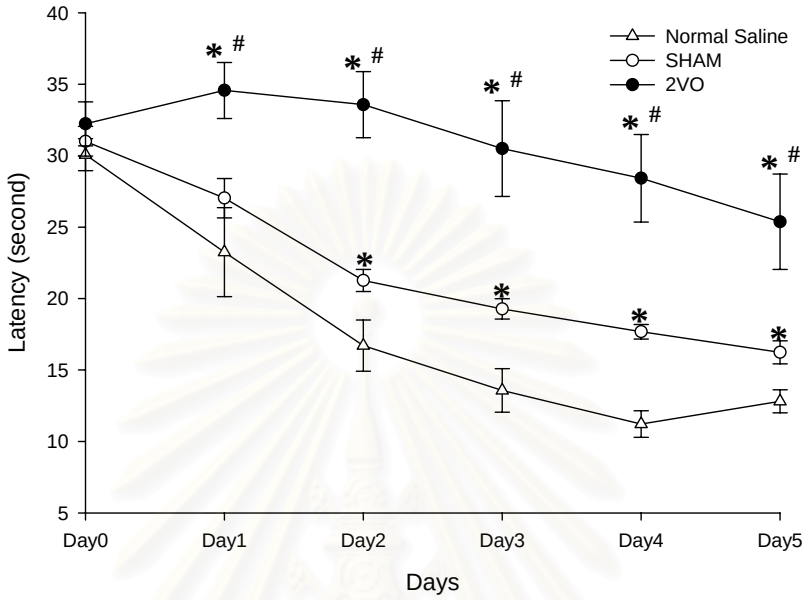

Figure 6 : Effects of 2VO-, Sham-operation, and normal saline on Morris water maze performance in mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in normal saline group.

#Significantly different from values in Sham-operated group.

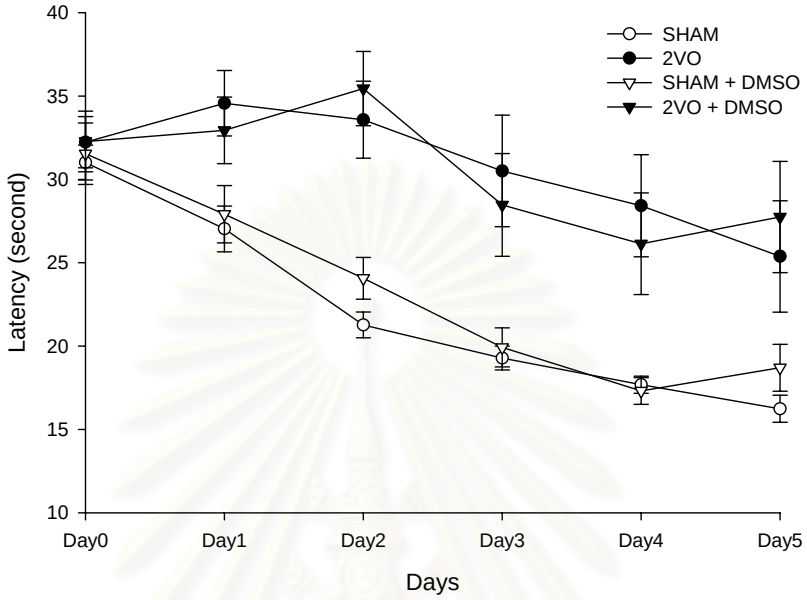

Figure 7 : Effects of treatment with DMSO on Morris water maze performance in 2VO- and Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons between groups. A significance level of $P < 0.05$ was considered as statistically significant difference.

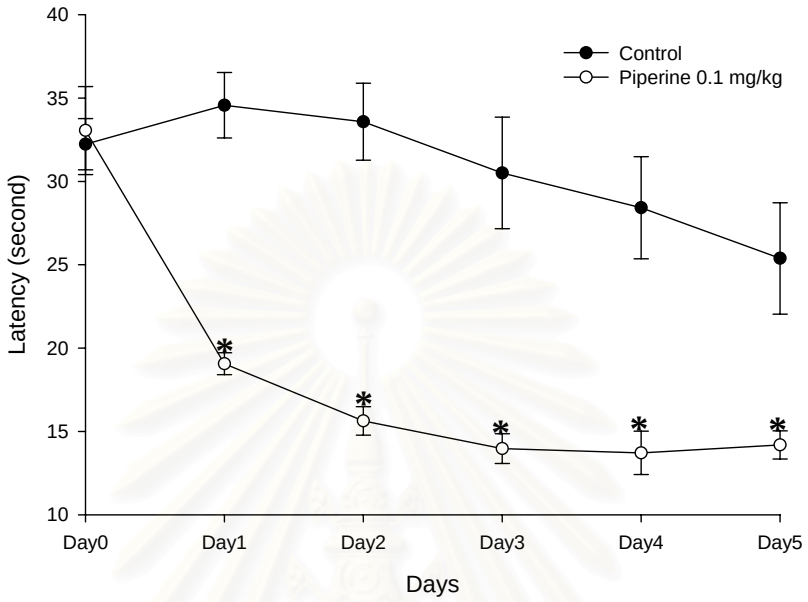

Figure 8 : Effects of treatment with 0.1 mg/kg piperine on Morris water maze performance in 2VO mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

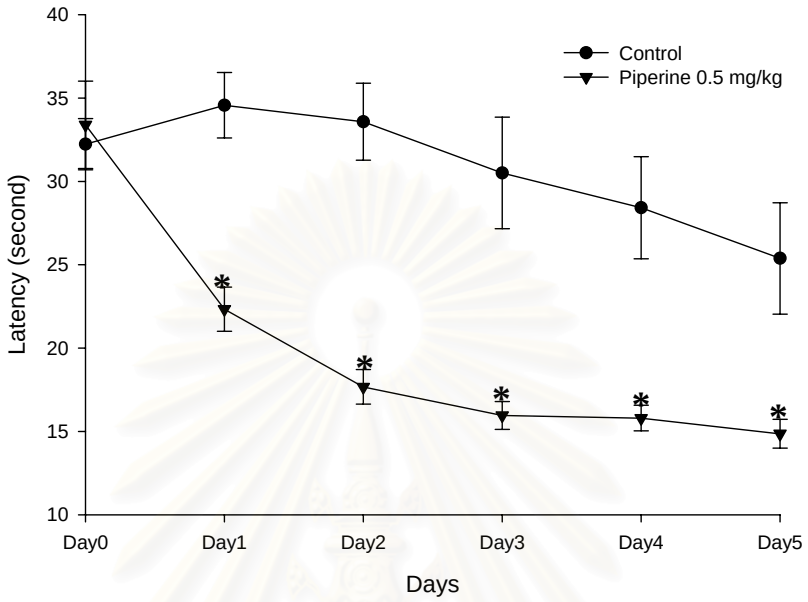

Figure 9 : Effects of treatment with 0.5 mg/kg piperine on Morris water maze performance in 2VO mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

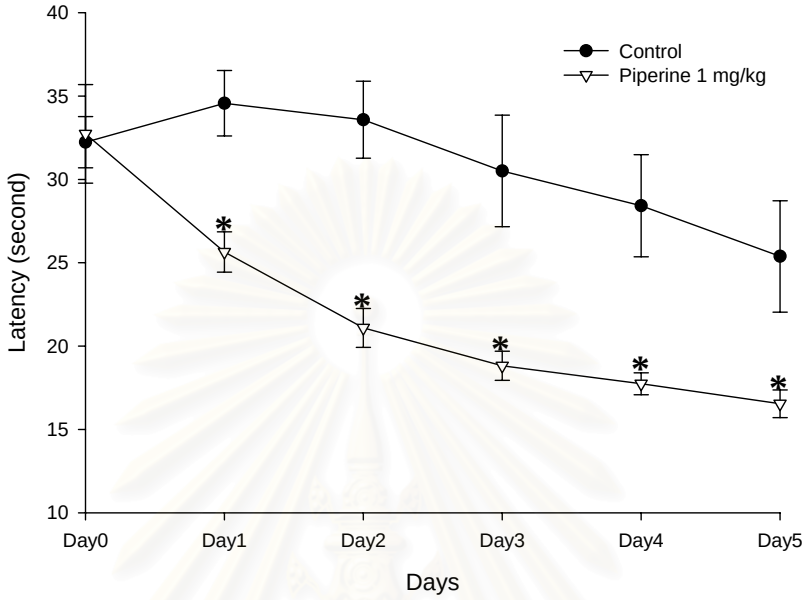

Figure 10 : Effects of treatment with 1 mg/kg piperine on Morris water maze performance in 2VO mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

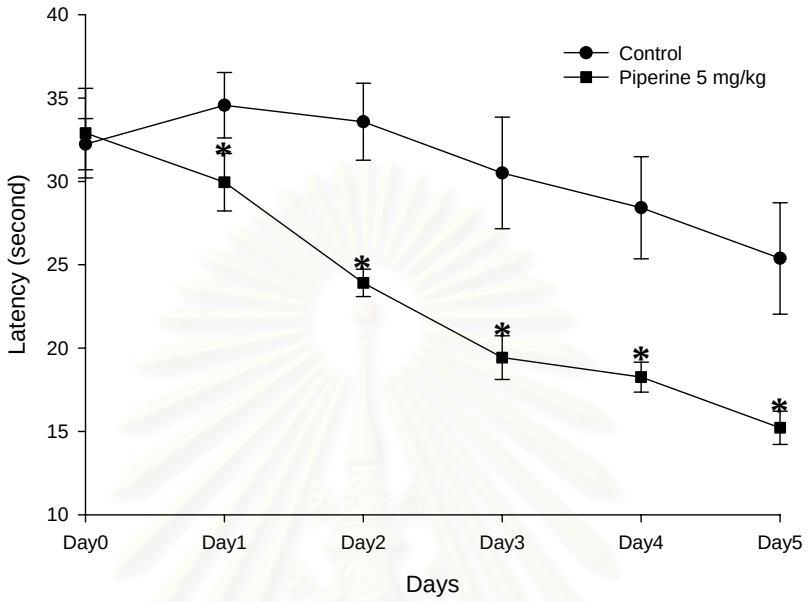

Figure 11 : Effects of treatment with 5 mg/kg piperine on Morris water maze performance in 2VO mice. The escape latency times are expressed as the mean ± SEM during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

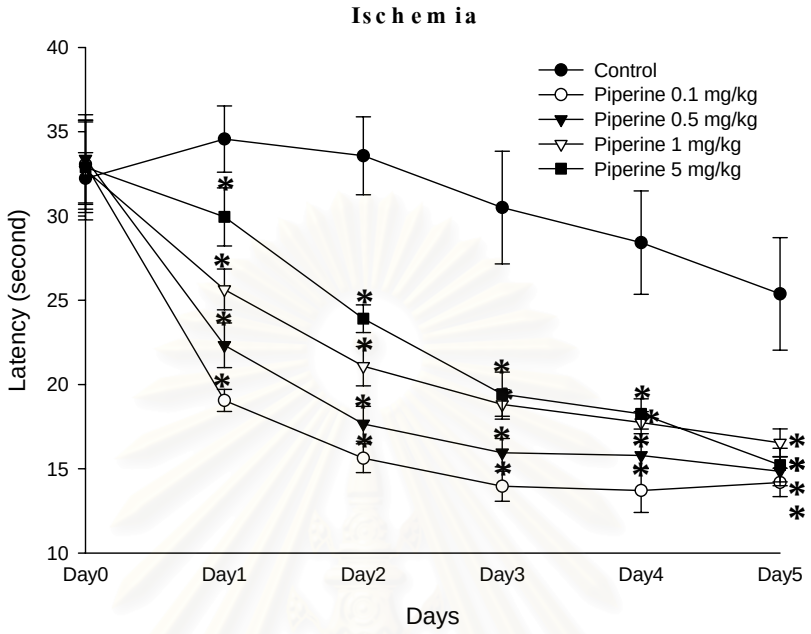

Figure 12 : Effects of treatment with various doses of piperine on Morris water maze performance in 2VO mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

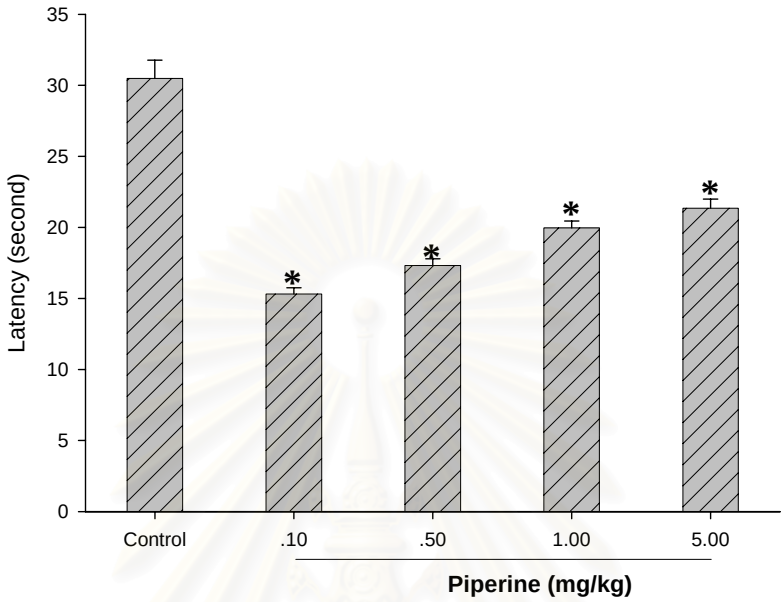

Figure 13 : Effects of treatment with various doses of piperine on Morris water maze performance in 2VO mice. The values are expressed as the mean ± SEM (n=8) of average escape latency times from 5 training days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

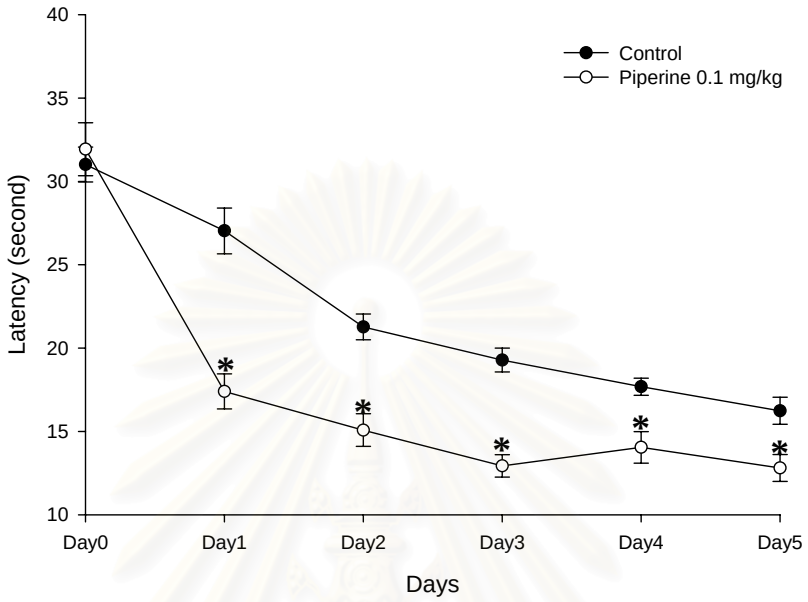

Figure 14 : Effects of treatment with 0.1 mg/kg piperine on Morris water maze performance in Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

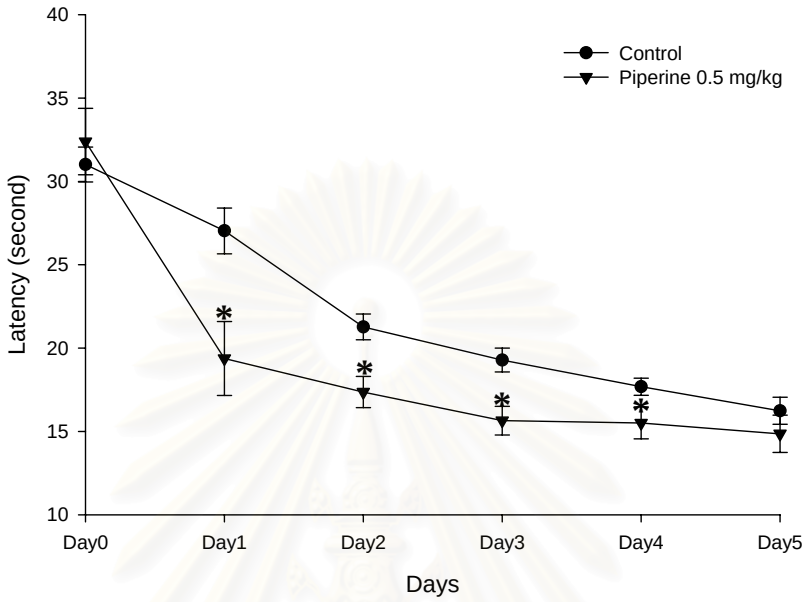

Figure 15 : Effects of treatment with 0.5 mg/kg piperine on Morris water maze performance in Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

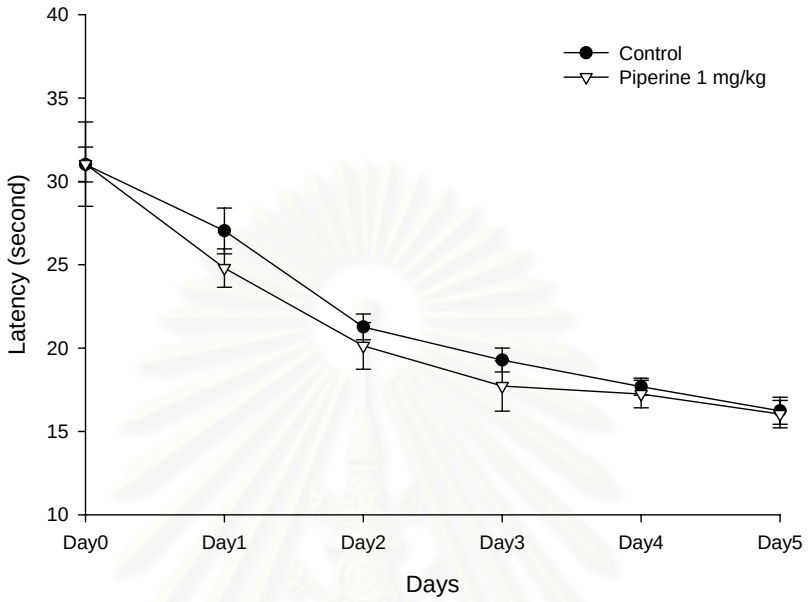

Figure 16 : Effects of treatment with 1 mg/kg piperine on Morris water maze performance in Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

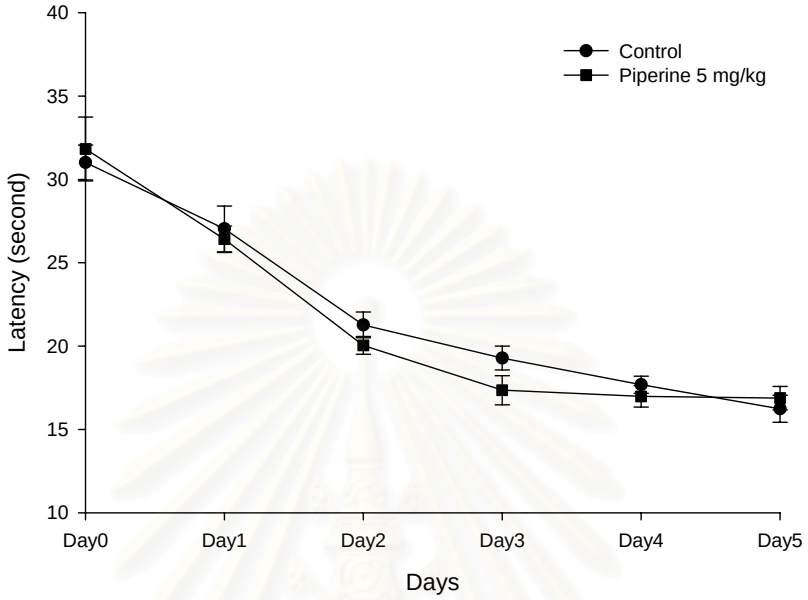

Figure 17 : Effects of treatment with 5 mg/kg piperine on Morris water maze performance in Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

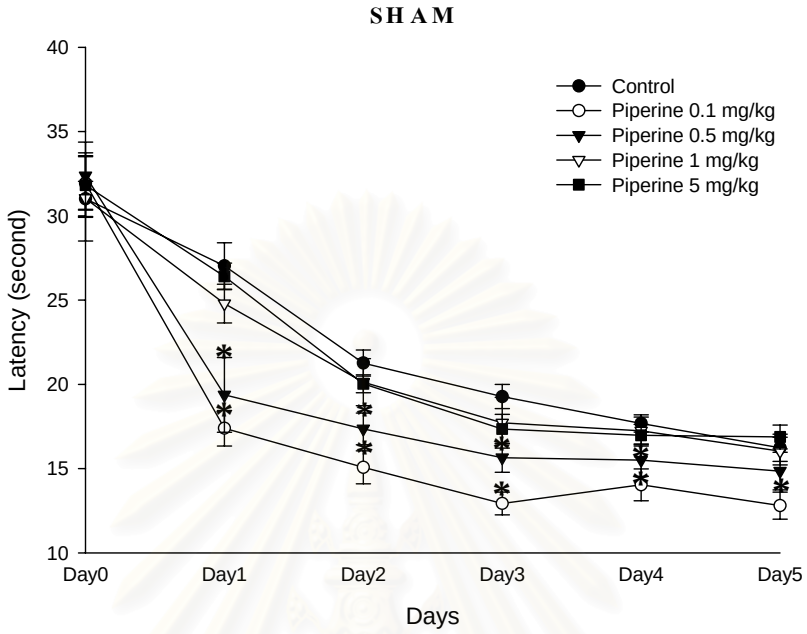

Figure 18 : Effects of treatment with various doses of piperine on Morris water maze performance in Sham-operated mice. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

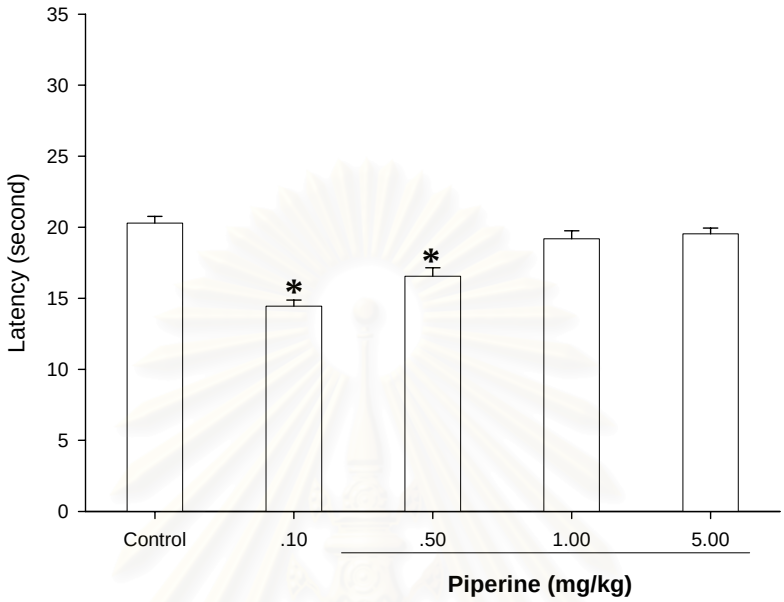

Figure 19 : Effects of treatment with various doses of piperine on Morris water maze performance in Sham-operated mice. The values are expressed as the mean ± SEM (n=8) of average escape latency times from 5 training days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) group.

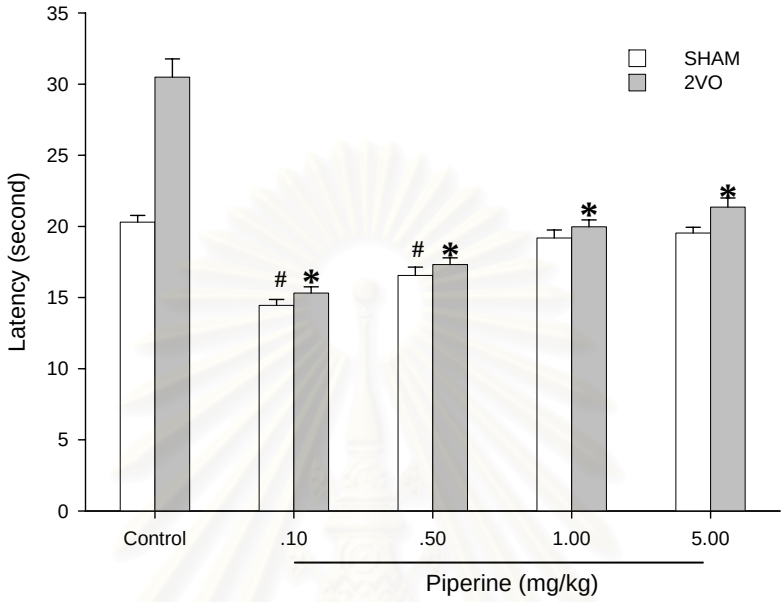

Figure 20 : Effects of treatment with various doses of piperine on Morris water maze performance in 2VO- and Sham-operated mice. The values are expressed as the mean ± SEM (n=8) of average escape latency times from 5 training days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

* Values in piperine-treated group are significantly different from values in control (DMSO-treated) groups – for 2VO mice.

Values in piperine-treated group are significantly different from values in control (DMSO-treated) groups – for Sham-operated mice.

Part IV: Effects of scopolamine on spatial learning and memory performance in mice.

Effects of scopolamine (a muscarinic cholinergic receptor antagonist) administration (0.5 and 1 mg/kg, i.p., 20 min before the water maze task) on water maze performance in mice are summarized in Figures 21 and 26, respectively. 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 training and persisted throughout the whole training schedule (5 days).

The average escape latency times of the scopolamine-treated mice (0.5 and 1 mg/kg, i.p.) were significantly higher than those in normal saline-treated group. (scopolamine 0.5 mg/kg = 33.73 ± 1.39 sec, $P < 0.05$; scopolamine 1 mg/kg = 37.44 ± 1.74 sec, $P < 0.05$ and normal saline group = 15.51 ± 8.63 sec). However, there was no statistically significant difference between effects of 0.5 and 1 mg/kg of scopolamine administration (Figures 31 and 32).

Part V: Effects of treatment with piperine on spatial learning and memory impairment in scopolamine-treated mice.

As shown in Figures 22, 23, 24, 25, 27, 28, 29, 30 and 32, administration of piperine at all test doses (0.1, 0.5, 1, and 5 mg/kg, i.p, 30-min before the water maze task) had no significant effect to scopolamine-induced amnesia in mice (0.5 and 1 mg/kg, i.p., 20 min before the water maze task). This suggested that piperine did not directly interact with the cholinergic system.

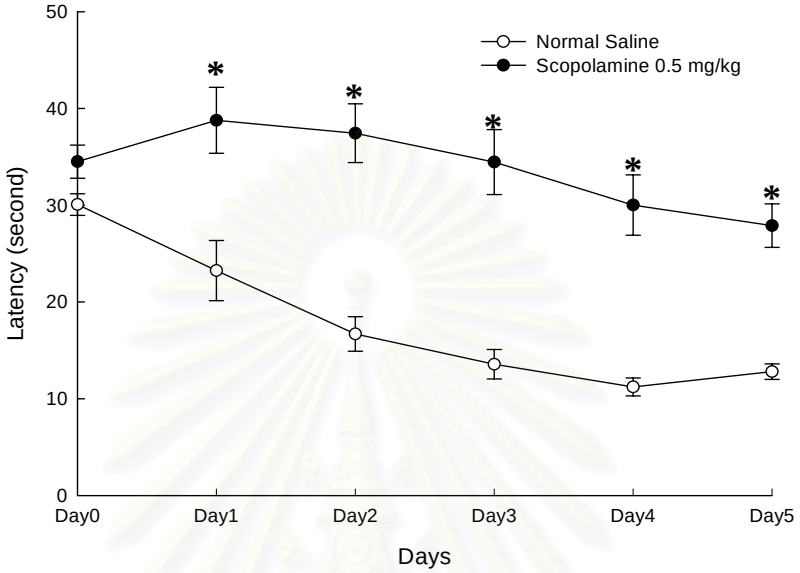

Figure 21 : Effects of scopolamine administration (0.5 mg/kg) on Morris water maze performance in mice. The escape latency times are expressed as the mean ± S.E.M (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (normal saline-treated) group.

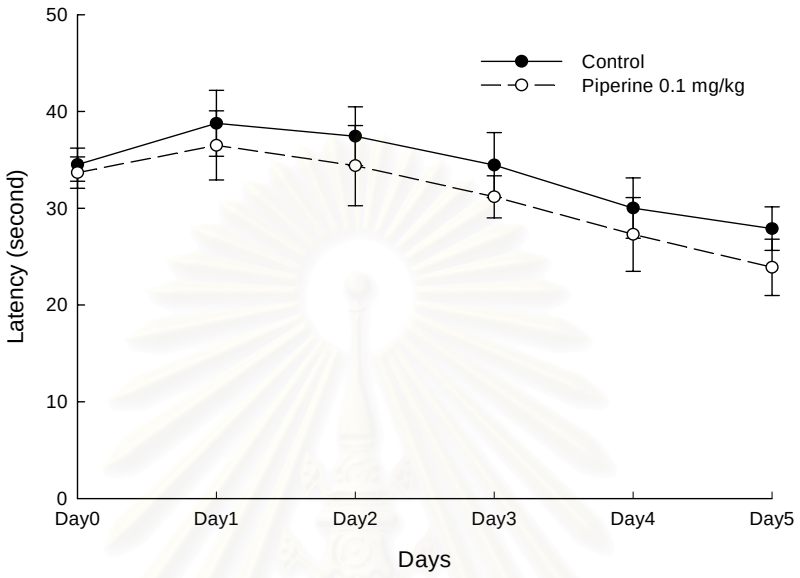

Figure 22 : Effects of treatment with 0.1 mg/kg piperine on scopolamine (0.5 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

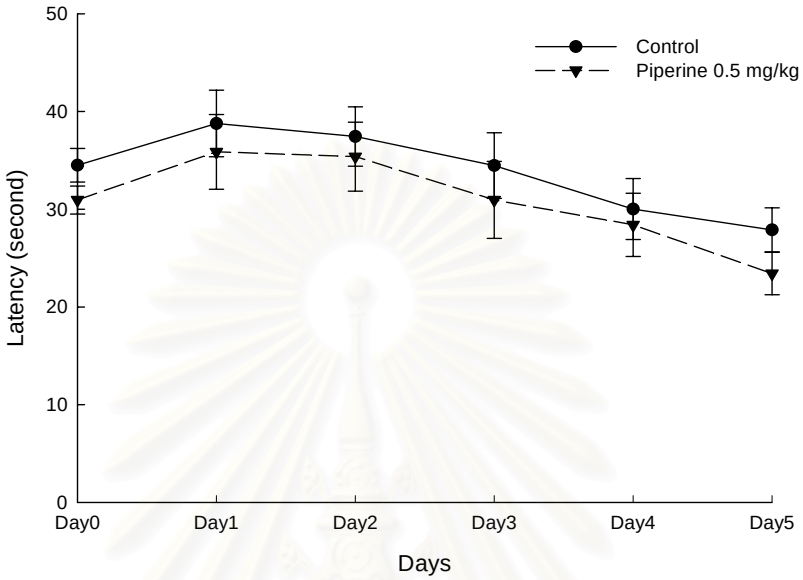

Figure 23 : Effects of treatment with 0.5 mg/kg piperine on scopolamine (0.5 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

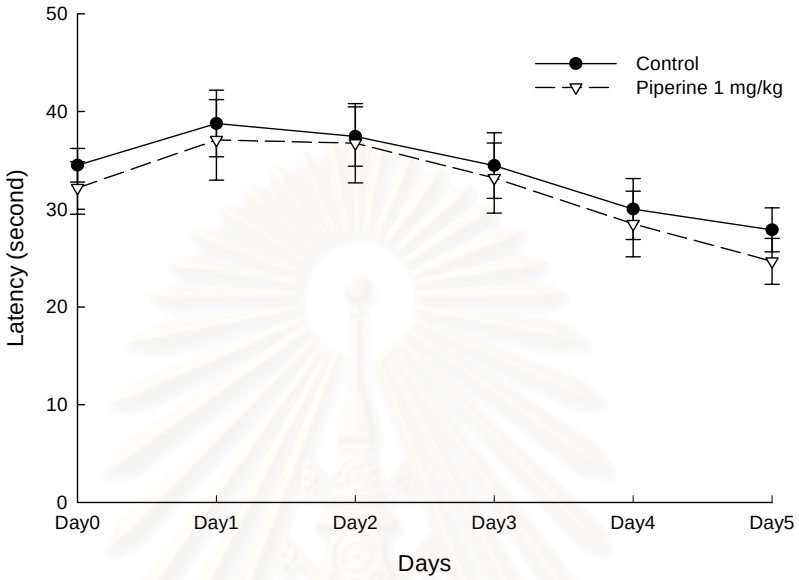

Figure 24 : Effects of treatment with 1 mg/kg piperine on scopolamine (0.5 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

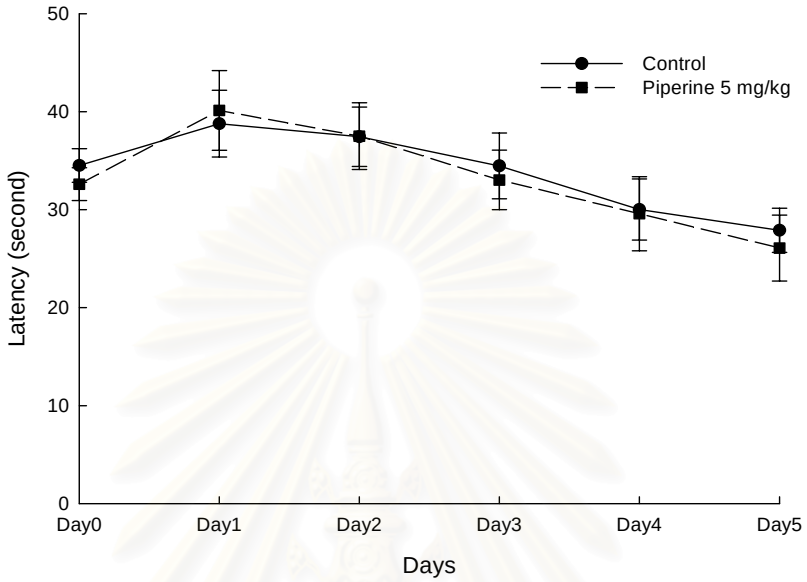

Figure 25 : Effects of treatment with 5 mg/kg piperine on scopolamine (0.5 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

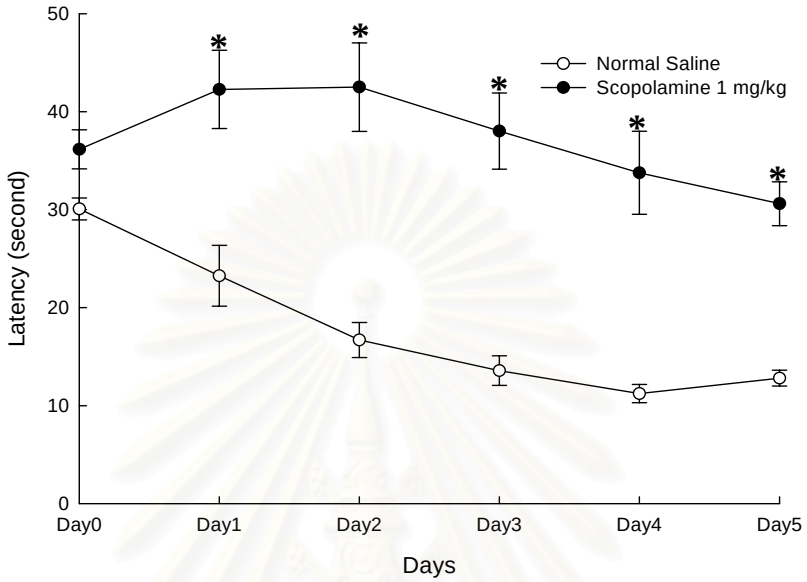

Figure 26 : Effects of scopolamine administration (1 mg/kg) on Morris water maze performance in mice. The escape latency times are expressed as the mean ± S.E.M (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (normal saline-treated) group.

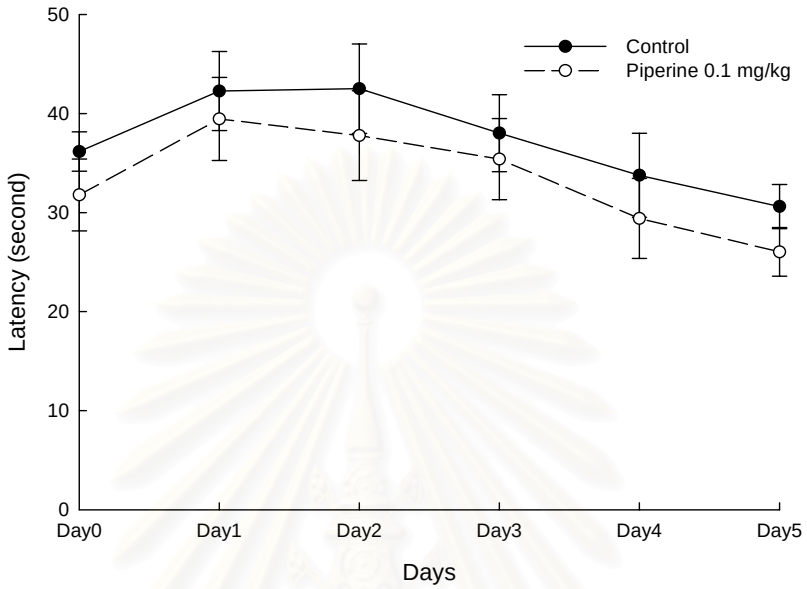

Figure 27 : Effects of treatment with 0.1 mg/kg piperine on scopolamine (1 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

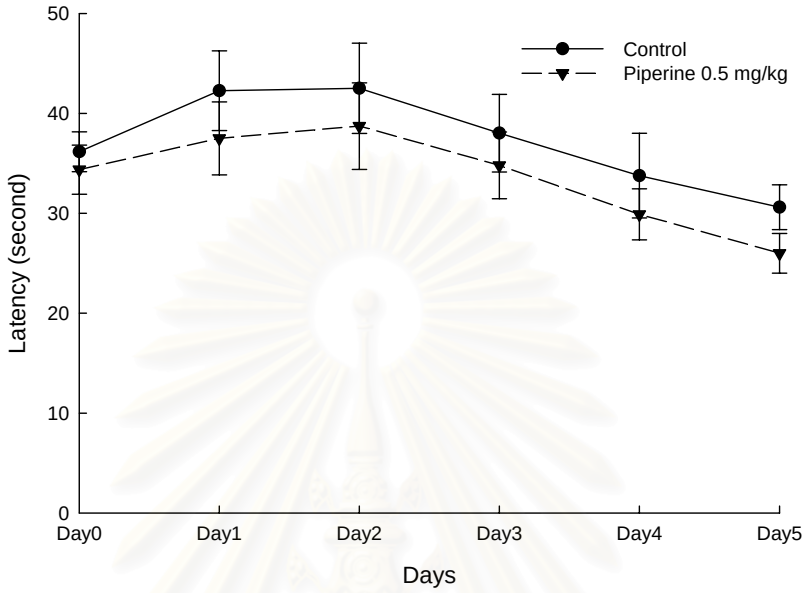

Figure 28 : Effects of treatment with 0.5 mg/kg piperine on scopolamine (1 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

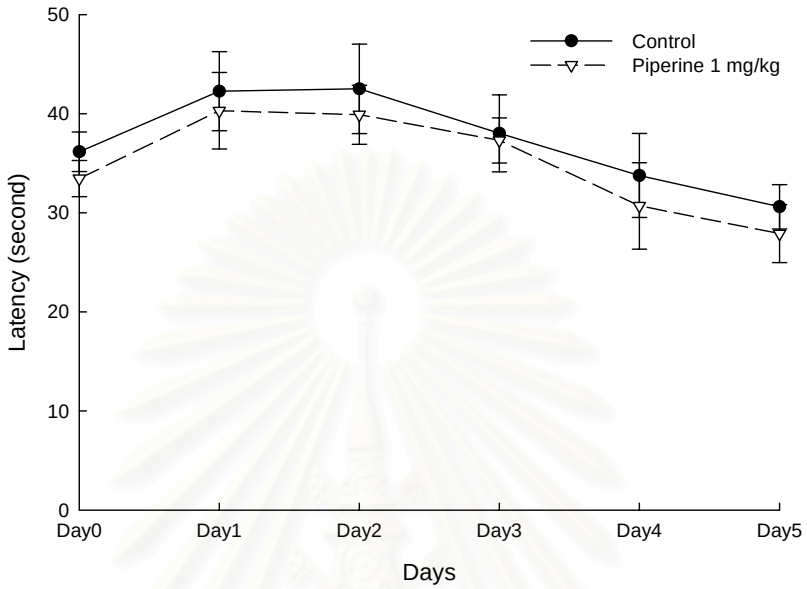

Figure 29 : Effects of treatment with 1 mg/kg piperine on scopolamine (1 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

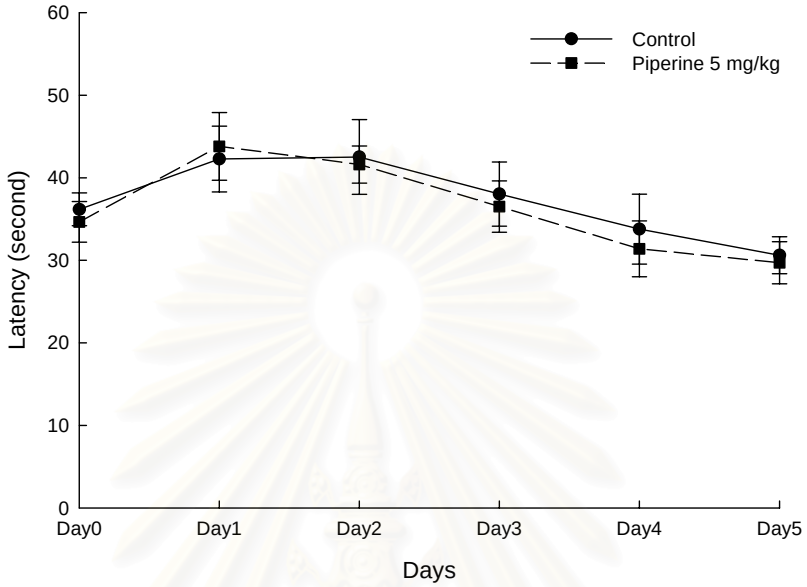

Figure 30 : Effects of treatment with 5 mg/kg piperine on scopolamine (1 mg/kg)-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. The escape latency times are expressed as the mean ± SEM (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

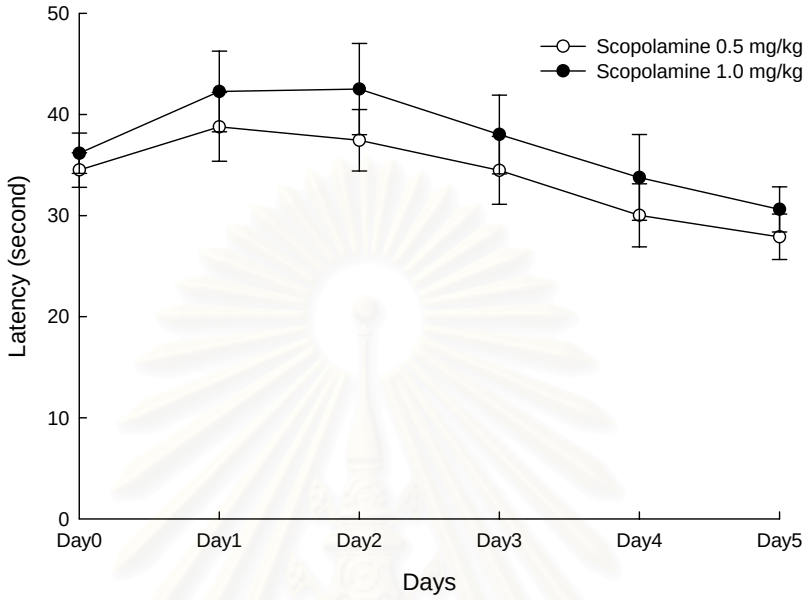

Figure 31 : Effects of scopolamine administration (0.5 and 1 mg/kg) on Morris water maze performance in mice. The escape latency times are expressed as the mean ± S.E.M (n=8) during a 5-day training schedule. A significance level of $P < 0.05$ was considered as statistically significant difference.

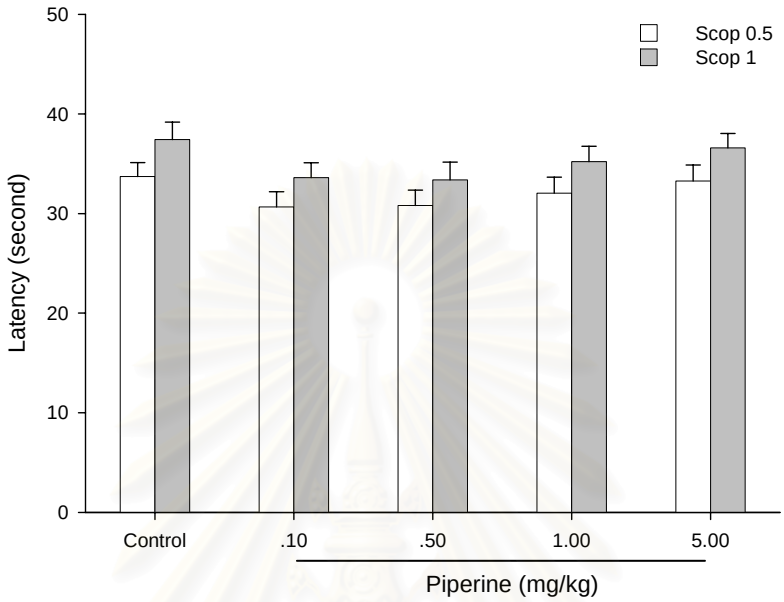

Figure 32 : Effects of treatment with piperine on scopolamine-induced amnesia in mice. Spatial learning and memory performance was tested in Morris water maze. Data are expressed as the mean ± SEM (n=8) of average escape latency times from 5 training days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

Part VI : Effects of piperine administration on locomotor activity in mice.

A. Effects of DMSO and normal saline administration on locomotor activity of mice.

Locomotor activities of mice after the administration of DMSO and normal saline are shown in Figure 33. In general, the motor activity (gross movements) of DMSO-treated mice was significantly lower than that of normal saline-treated mice. At certain time points (15, 30, 35, 40 and 45 min), the differences were statistically significant. Considering total movement counts during a 60-min test period, the value from DMSO-treated mice was significantly lower than that from normal saline-treated mice. (365.63 ± 24.75 vs 779.00 ± 56.71; $p < 0.05$). These results suggested that DMSO administration might depress the motor activity of mice.

B. Effects of piperine administration on locomotor activity in mice.

As shown in Figures 34, 35, 36, 37 and 38, piperine administration at various doses (0.1, 0.5, 1, and 5 mg/kg, i.p.) did not show any apparent effects on locomotor activity of mice as compared to control (DMSO-treated) group. In addition, there were no significant effects of piperine on the total movement counts among all piperine-treated groups and control group (Figure 39). It appeared that piperine, at doses of 0.1 to 5 mg/kg, had no significant effects on the motor activity of mice.

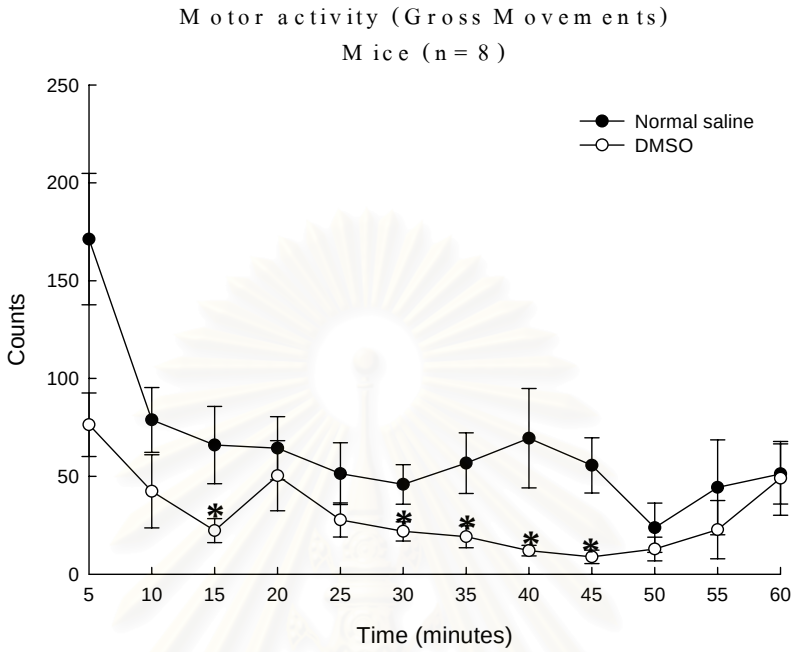

Figure 33 : Effects of DMSO and normal saline administration on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in normal saline-treated group.

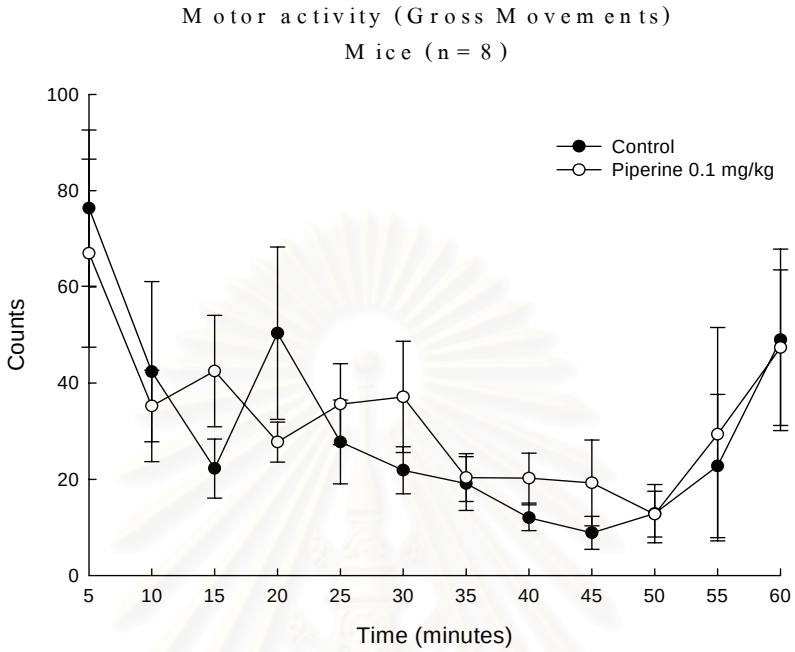

Figure 34 : Effects of piperine administration (0.1 mg/kg) on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. A significance level of $P < 0.05$ was considered as statistically significant difference.

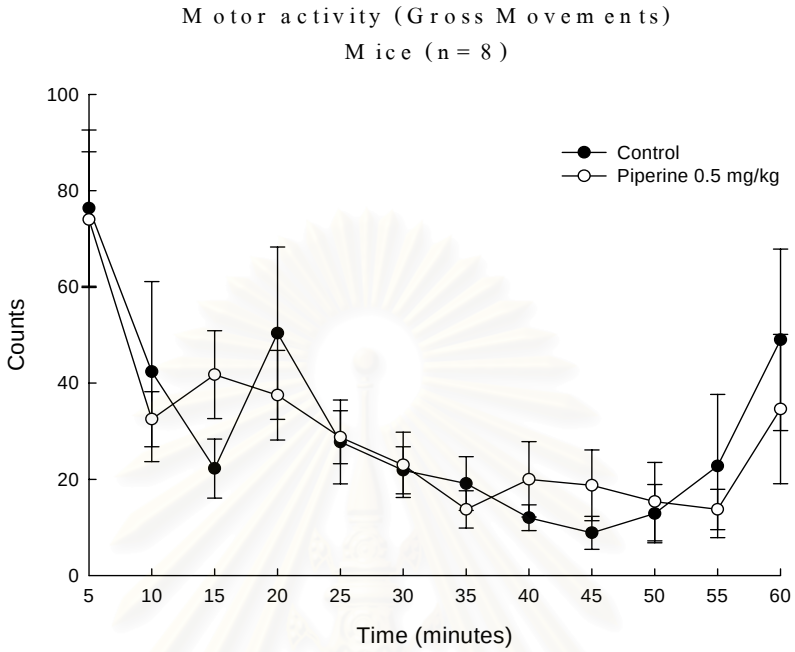

Figure 35 : Effects of piperine administration (0.5 mg/kg) on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. A significance level of $P < 0.05$ was considered as statistically significant difference.

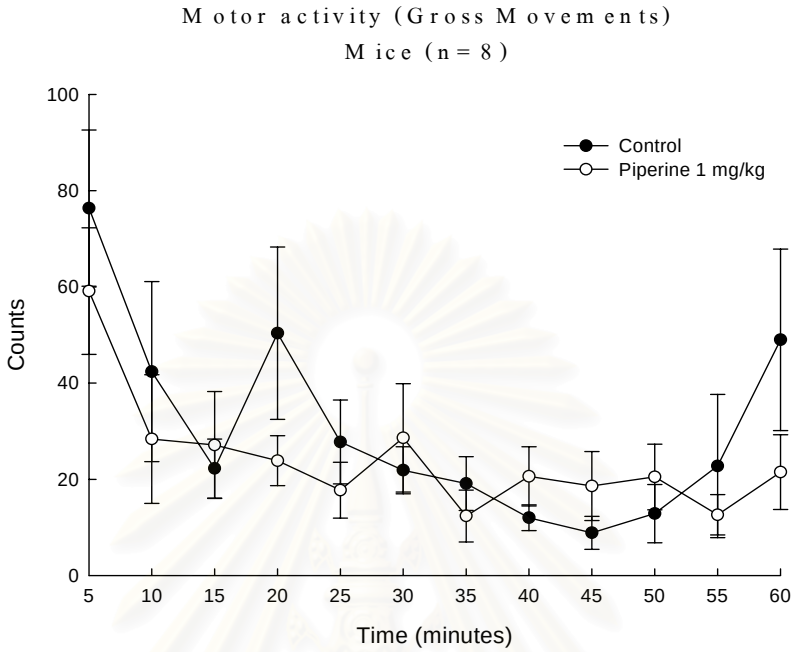

Figure 36 : Effects of piperine administration (1 mg/kg) on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. A significance level of $P < 0.05$ was considered as statistically significant difference.

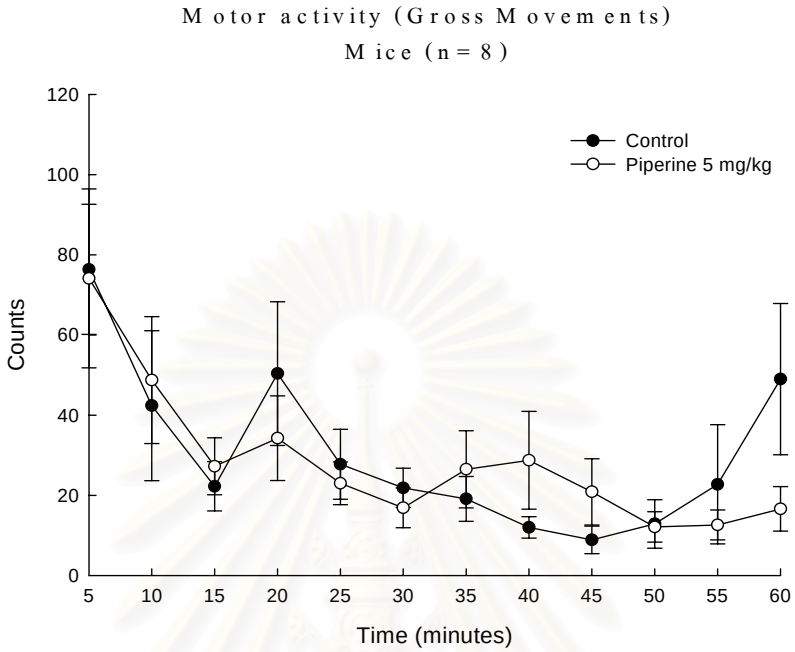

Figure 37 : Effects of piperine administration (5 mg/kg) on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. A significance level of $P < 0.05$ was considered as statistically significant difference.

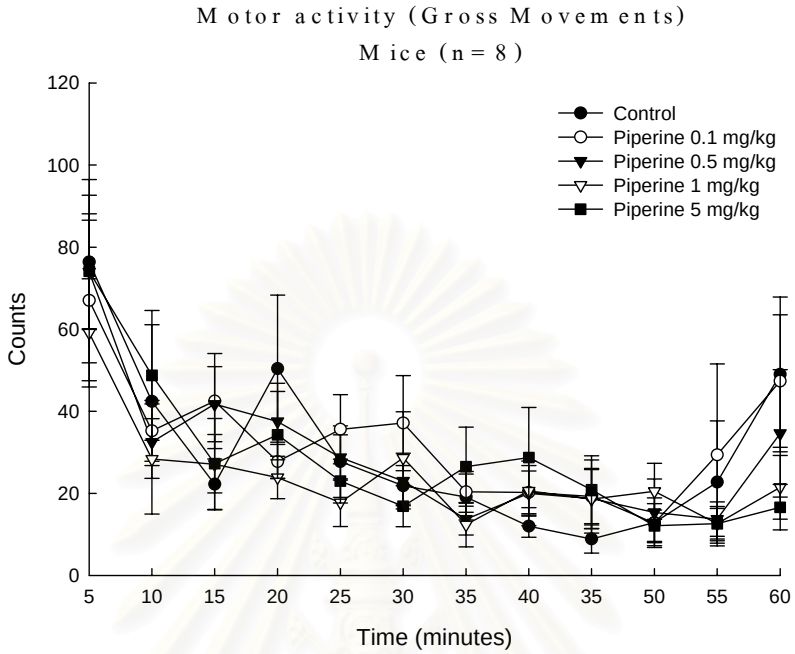

Figure 38 : Effects of piperine administration at various doses on locomotor activity in mice. Data are expressed as the mean ± SEM (n=8) of registered counts of motor activity (gross movements) every 5-min interval during a 60-min test period. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

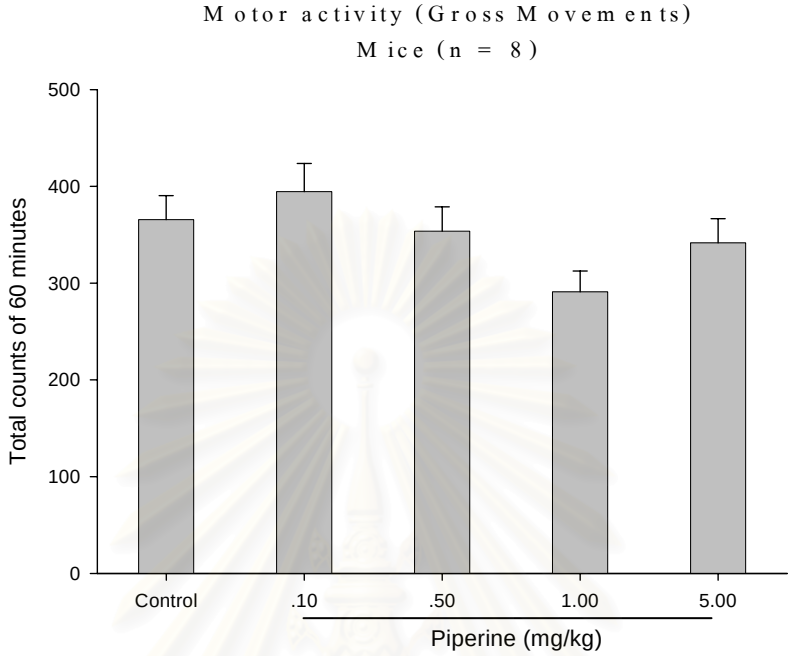

Figure 39 : Effects of piperine administration at various doses on locomotor activity in mice. Data are expressed as the mean ± SEM (N=8) of total counts of motor activity (gross movements) during a 60-min test period. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

Part VII : Effects of piperine administration on brain lipid peroxidation in mice after transient cerebral ischemia.

A. Effects of 2VO- and Sham-operation on brain lipid peroxidation.

The brain levels of TBARS, an indication of lipid peroxidation, in mice at 5 days after 2VO- and Sham-operated procedure are shown in Figure 40. Both procedures caused significant increases in brain lipid peroxidation as compared to normal animals. In addition, 2VO operation induced a marked and significantly higher increase in brain lipid peroxidation than Sham operation. Therefore, brain injuries caused by surgical process and transient cerebral ischemia could induce oxidative stress in the animal brain that led to an impairment in spatial learning and memory.

B. Effect of DMSO administration on brain lipid peroxidation.

The TBARs brain levels of 2VO- and Sham-operated mice after 5-day administration of normal saline or DMSO were shown in Figure 41. No significant difference between TBARs levels of mice treated with normal saline and DMSO was found in both 2VO- and Sham-operated groups.

C. Effects of piperine administration on brain lipid peroxidation in mice after transient cerebral ischemia.

The protective effect of piperine treatment on brain lipid peroxidation induced by cerebral ischemia in mice was shown in Figure 46. At 5 days following transient cerebral ischemia and Sham operation, TBARs levels of the brain homogenates increased to 37.71 ± 1.80 nmol/g tissue and 26.60 ± 0.97 nmol/g tissue, respectively, as compared to of untreated control brain (21.99 ± 1.41 nmol/g tissue). Piperine administration at the dose of 0.1 mg/kg/day for 5 days after cerebral ischemia and Sham-operation markedly attenuated TBARs levels to 22.37 ± 1.49 nmol/g tissue and 20.29 ± 1.06 nmol/g tissue, respectively (Table 12, Figure 42). Administration of piperine at doses of 0.5 and 1 mg/kg/day for 5 days

significantly decreased only TBARs levels after cerebral ischemia but not after Sham operation (Figure 43 and 44).

However, administration of piperine at the dose of 5 mg/kg/day for 5 days demonstrated no significant effects on TBARs levels after both cerebral ischemia and Sham operation (Figure 45).

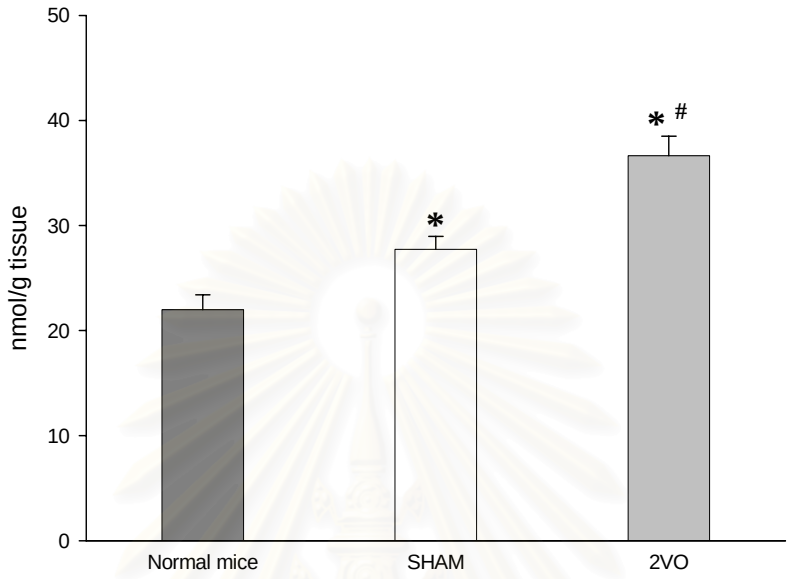

Figure 40 : Effects of transient cerebral ischemia and Sham operation on brain levels of thiobarbituric acid reactive substances (TBARs). The operation was made and TBARs assay was done at 5 days after. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in normal mice group.

#Significantly different from values in Sham-operated group.

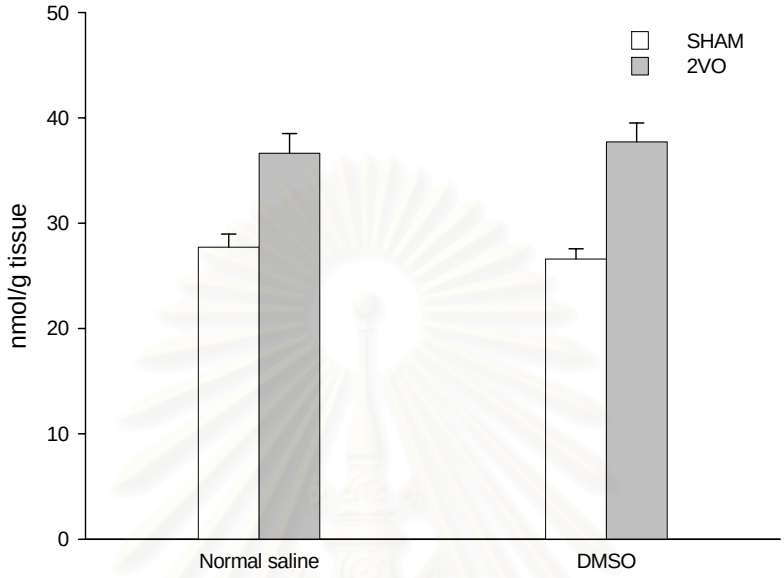

Figure 41 : Effects of normal saline and DMSO administration on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with normal saline or DMSO once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

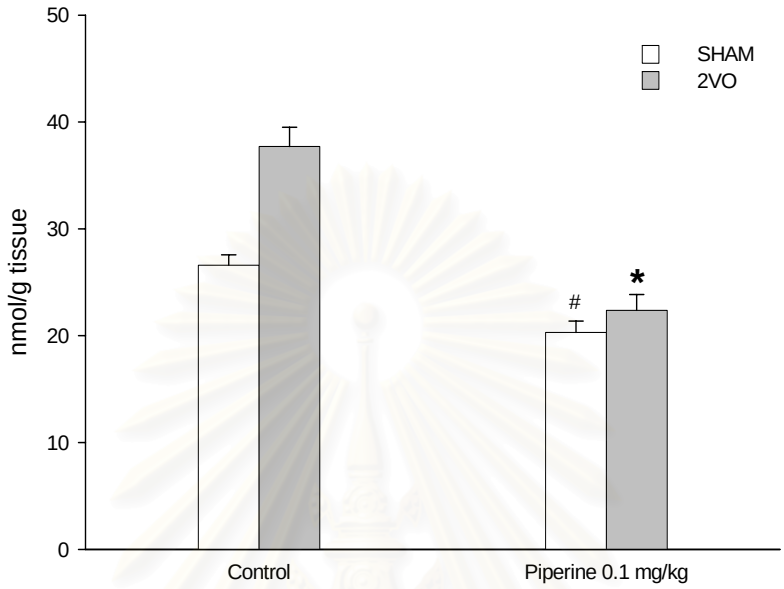

Figure 42 : Effects of piperine administration (0.1 mg/kg) on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with piperine or DMSO once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) cerebral ischemia group.

#Significantly different from values in control (DMSO-treated) sham-operated group.

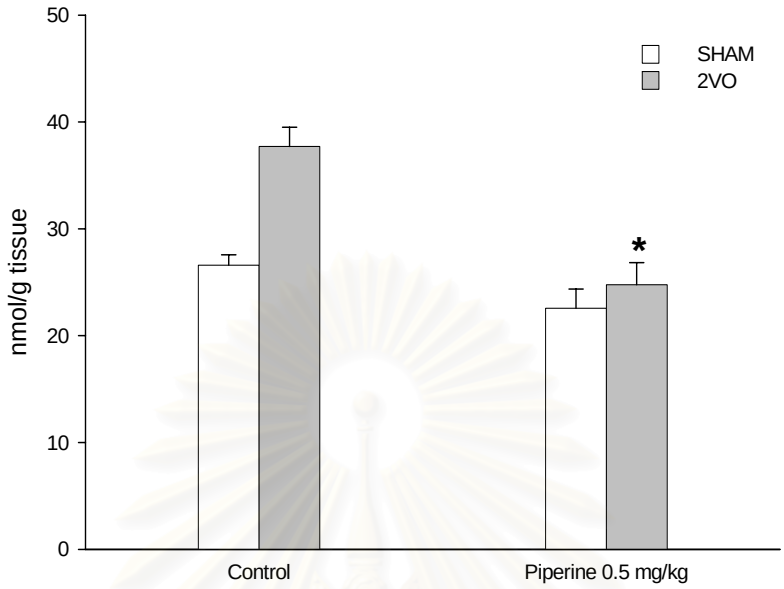

Figure 43 : Effects of piperine administration (0.5 mg/kg) on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with piperine or DMSO once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) cerebral ischemia group.

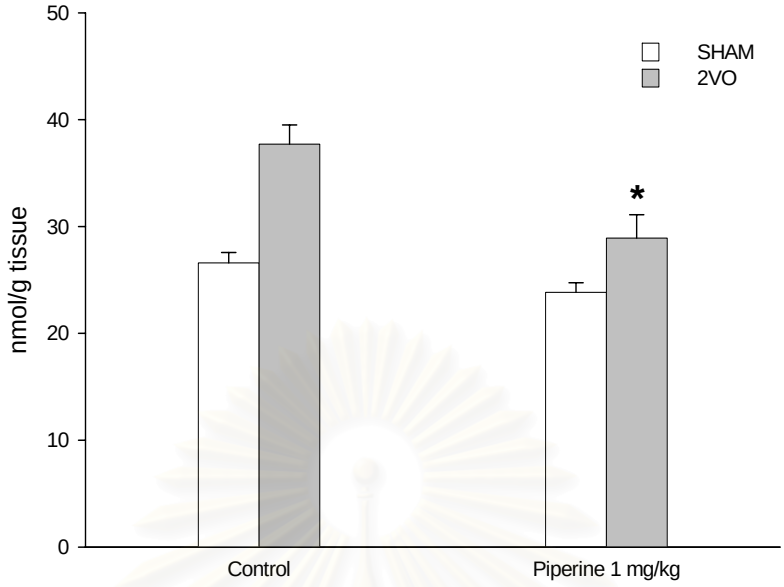

Figure 44 : Effects of piperine administration (1 mg/kg) on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with piperine or DMSO once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

*Significantly different from values in control (DMSO-treated) cerebral ischemia group.

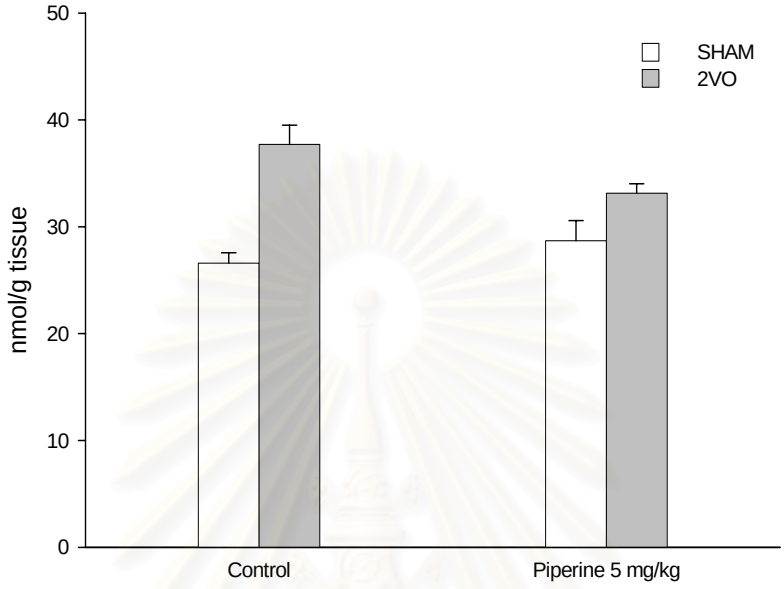

Figure 45 : Effects of piperine administration (5 mg/kg) on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with piperine or DMSO once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM (n=4) of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

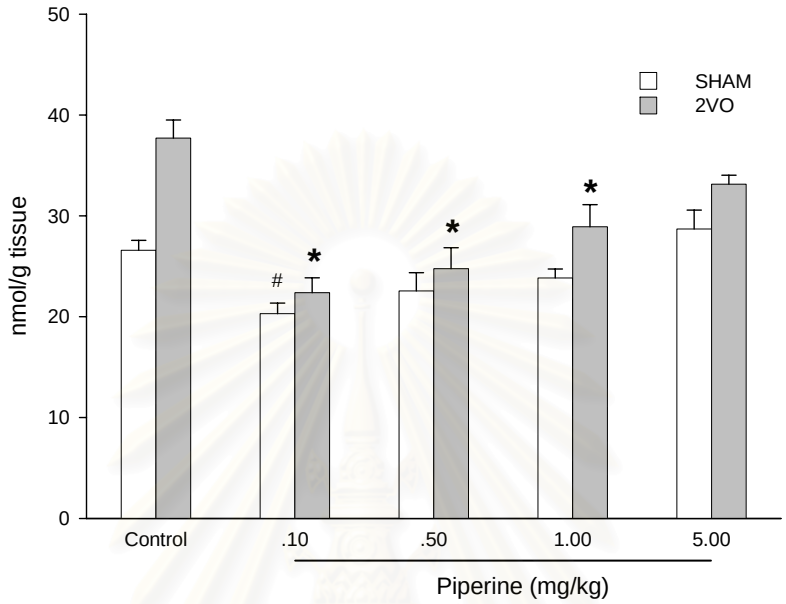

Figure 46 : The comparison of effects of piperine administration at various doses on brain levels of thiobarbituric acid reactive substances (TBARs) after transient cerebral ischemia and Sham operation in mice. Following the operation, mice were i.p. administered with piperine (0.1, 0.5, 1 and 5 mg/kg, i.p.) or DMSO (0.1 ml, i.p) once daily for 5 consecutive days, prior to TBARs assay. Values are expressed as the mean ± SEM of TEP amount in nmol/g tissue. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons. A significance level of $P < 0.05$ was considered as statistically significant difference.

[*]Significantly different from values in control (DMSO-treated) cerebral ischemia group.

[#]Significantly different from values in control (DMSO-treated) Sham-operated group.

Chapter 4

Discussion

This study provides the first evidence for the possible beneficial effects of piperine on learning and memory impairment and brain lipid peroxidation increase induced by transient cerebral ischemia in mice. Intraperitoneal administration of piperine, the pungent alkaloid found in pepper, at lower doses (0.1 and 0.5 mg/kg) for 5 consecutive days, improved spatial learning and memory performance of mice in Morris water maze. This beneficial effect of piperine on memory function was nullified at doses of 1 and 5 mg/kg and became deteriorative at higher doses of 10 and 15 mg/kg. This phenomenon could be explained by a known chemical property of piperine; it behaved as antioxidant at low concentrations and behaved as prooxidant at high concentrations. This bimodal property is a common characteristic of many natural antioxidants.

Accordingly, piperine at a lower dose range from 0.1 to 5 mg/kg/day was selected for further study for its beneficial effects on memory function and brain lipid peroxidation. However, a higher dose range of 10 to 15 mg/kg/day was not selected for further study because it might exert some toxic effects which may complicate the experimental outcome. In this connection, it had been reported that piperine at a high concentration of 100 μM was cytotoxic to cultured neurons from the embryonic rat brain (Unchern *et al*., 1997).

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). These neurodegenerative processes led to marked deficits in many brain functions including learning and memory. After the 2VO procedure, mice with transient cerebral ischemia showed marked impairment of spatial learning and memory performance in Morris water maze. They slowly learned to find the hidden platform (increased escape latency) and spent more time swimming around the pool throughout the 5-day training schedule. The Sham-operated mice which

received only surgical maneuver without carotid artery occlusion also showed a certain degree of learning and memory impairment although it was less severe than that of the 2VO mice. This is explainable by the effect of brain trauma induced by surgical process.

The daily intraperitoneal (i.p.) administration of piperine at various doses (0.1, 0.5, 1 and 5 mg/kg/day, 30 min before the water maze task, for 5 consecutive days) revealed beneficial effects on spatial learning and memory impairment in both Sham-operated and 2VO mice. The attenuation of memory deficits was remarkable in 2VO mice in such a way that all piperine doses tested were effective. The beneficial effect of piperine found in Sham-operated mice was marginal because only doses of 0.1 and 0.5 mg/kg were effective.

It was apparent that piperine was more effective at preventing memory deficit at lower doses than higher doses. This particular dose-response characteristic may be due to the bimodal property of piperine (antioxidant/prooxidant) depending on its concentrations at the site of action. Moreover, there might be other unknown mechanisms underlying this unique effect of piperine on memory functions. Another point of interest was the remarked beneficial effects of piperine on ischemic brain at lower doses. This may be advantageous and practical for further study and application in the management of neurodegenerative disorders under actual clinical settings.

Several studies have provided evidence for the possible involvement of the cholinergic system in learning and memory function (Beninger et al., 1989; New house, 1990). As shown in the introduction section, previous studies also supported this idea in terms of the correlation of the acetylcholine levels, and choline acetyltransferase and cholinesterase activities with dementia of Alzheimer's disease and cerebrovascular disease (Bartus et al., 1982; Coyle et al., 1983). In this study, piperine administration at all test doses had no beneficial effects on spatial memory impairment induced by scopolamine administration. This finding suggested that beneficial effects of piperine on memory functions in mice with transient cerebral ischemia might not involve the central cholinergic system. Up to now, there has been no reported evidence for cholinomimetic property of piperine.

As described in the Results section, piperine had no stimulatory effects on locomotor activity of mice at all test doses. This finding implied that beneficial effects of piperine on spatial learning and memory impairment were unlikely to involve the improvement of motor function or activity. Piperine may act through yet undefined mechanisms in the brain thereby improving the learning and memory performance.

Free radical species of potential importance were markedly generated in the brain after transient cerebral ischemia and reperfusion. Brain lipid peroxidation was also initiated and, in combination with other hazardous free radical species, led to brain injuries, neuronal damage, and impairment of memory functions. The brain lipid peroxidation (as measured by TBARs assay) of 2VO-mice at 5 days after the occlusion was significantly increased when compared to Sham-operated mice. Administration of piperine at doses of 0.1 and 0.5 mg/kg/day, i.p., for 5 consecutive days, significantly prevented brain lipid peroxidation increase by cerebral ischemia while the administration of piperine at higher dose (1 mg/kg/day) showed modest attenuation on the increase. However, preventive effect of piperine was nullified at the highest dose tested (5 mg/kg/day).

Preventive effect of piperine on brain lipid peroxidation increase in Sham-operated mice was also noticeable. However, only piperine administration at a dose of 0.1 mg/kg/day showed significant beneficial effect on brain lipid peroxidation whereas the other three test doses (0.5, 1 and 5 mg/kg/day) did not show any effects. These findings suggested that in normal to moderate oxidative stress condition, piperine may not play an important role in antioxidative capacity of the brain.

Antioxidant property of piperine had been well established. Koul *et al*. (1993) suggested that piperine exerted a significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity by reducing both in *in vitro* and *in vivo* lipid peroxidation, enzymatic leakage of GPT and AP, and by preventing the depletion of GSH and total thiols in the intoxicated mice.

The close correlation between beneficial effects of piperine on spatial learning and memory impairment and brain lipid peroxidation suggests that these

two effects may be related. It is possible that the mechanism by which piperine improves memory deficit in mice with cerebral ischemia might involve, at least partly, reduction of lipid peroxidation in neuronal membranes via antioxidant property of piperine. However, other mechanisms are also possible, such as the stimulatory effect of piperine on neuronal/glial metabolism or functions. The actual underlying mechanisms of action of piperine on memory functions are still awaiting for further investigations.

Chapter 5

Conclusion

In this study, effects of piperine, a major pungent alkaloid in pepper, on spatial learning and memory impairment and brain lipid peroxidation increase were investigated. Transient cerebral ischemia induced by bilateral common carotid artery occlusion and scopolamine administration were used as the cognitive testing models. The experimental results obtained in this study lead to the conclusion as follows:

- Piperine administration had beneficial effects on 2VO-induced cognitive deficit and brain lipid peroxidation increase in mice.
- The close correlation between effects of piperine on both indications of brain injury also implied that the attenuation of 2VO-induced cognitive deficit may involve, at least partly, the antioxidant property of piperine.
- Conceivably, piperine may be considerable for further study as a possible adjunctive medication in the treatment of neurodegenerative disorders.

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APPENDICES

Effects of piperine, DMSO & NSS treatment on performance of mice in spatial memory task. → Effective dose finding. (5 days)

Group name		Escape latency in the Morris water-maze task					Mean
	n	Day1	Day2	Day3	Day4	Day5	Days 1-5
Normal Saline	8	23.25 ± 3.11	16.71 ± 1.79	13.57 ± 1.52	11.22 ± 0.93	12.81± 0.80	15.51 ± 0.86
DMSO	8	25.86 ± 1.36	21.07 ± 1.06	17.56 ± 0.69	15.03 ± 0.63	14.33 ± 0.73	18.77 ± 0.51 *
Piperine 0.1 mg/kg	8	16.60 ± 1.30	14.92 ± 1.29	12.43 ± 0.99	13.08 ± 0.93	10.54 ± 0.58	13.51 ± 0.49 #
Piperine 0.5 mg/kg	8	17.34 ± 1.15	15.36 ± 1.09	13.05 ± 1.05	12.24 ± 0.82	11.04 ± 0.73	13.81 ± 0.46 #
Piperine 1 mg/kg	8	26.93 ± 2.64	19.18 ± 2.40	14.87 ± 0.97	12.80 ± 0.84	11.02 ± 0.64	16.96 ± 0.86
Piperine 5 mg/kg	8	27.48 ± 3.48	20.13 ± 2.32	15.03 ± 1.05	13.76 ± 1.20	11.20 ± 0.65	17.52 ± 0.98
Piperine 10 mg/kg	8	32.77 ± 3.94	21.94 ± 2.01	20.66 ± 2.94	23.80 ± 3.86	19.07 ± 2.75	23.65 ± 1.45 *#
Piperine 15 mg/kg	8	34.27 ± 4.56	26.53 ± 3.98	29.50 ± 4.13	28.46 ± 3.62	26.16 ± 3.69	28.98 ± 1.79 *#

Table 1 : The effect of pretreatment with different doses of Piperine (0.1, 0.5, 1, 5, 10, and 15 mg/kg, i.p.) on performance of mice in the Morris water-maze. The values are expressed as the mean ± S.E.M of the escape latency for 5 consecutive days. Statistical analyses were performed by one-way ANOVA and Tukey HSD post-hoc test for planned comparisons between without and with Piperine treatment. A significance value of P less than 0.05 ($P < 0.05$) was considered as statistically significant. *Significantly different from value in Normal saline treatment group. #Significantly different from value in control group (DMSO).

Table 2 : The performance of mice in spatial memory task of 2VO group compared with Sham-operated group during the experiment of 8 days.

	SHAM			2VO		
	Mean		SEM	Mean		SEM
Day0	31.01	±	1.05	32.23	±	1.54
Day1	27.03	±	1.38	34.57 *	±	1.96
Day2	21.27	±	0.78	33.57 *	±	2.31
Day3	19.28	±	0.71	30.50 *	±	3.35
Day4	17.68	±	0.51	28.42 *	±	3.06
Day5	16.24	±	0.81	25.38 *	±	3.34
Day6	16.13	±	0.66	15.68	±	1.54
Day7	16.96	±	0.95	15.09	±	1.66
Day8	14.11	±	0.81	15.39	±	2.20
Mean	20.03	±	0.47	30.49 *	±	1.29

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

* P<0.05; Significant difference between Cerebarl ischemia group vs. Sham operation group.

Table 3 : The escapes latency of DMSO treatment groups compared with normal saline treatment groups.

	Normal saline						DMSO Treatment					
	SHAM			2VO			SHAM			2VO		
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Day0	31.01	±	1.05	32.23	±	1.54	31.54	±	1.84	32.28	±	1.82
Day1	27.03	±	1.38	34.57	±	1.96	27.91	±	1.72	32.94 #	±	2.00
Day2	21.27	±	0.78	33.57	±	2.31	24.06	±	1.25	35.45 #	±	2.23
Day3	19.28	±	0.71	30.50	±	3.35	19.92	±	1.17	28.46 #	±	3.08
Day4	17.68	±	0.51	28.42	±	3.06	17.31	±	0.80	26.14 #	±	3.05
Day5	16.24	±	0.81	25.38	±	3.34	18.70	±	1.41	27.74 #	±	3.33
Mean	20.30	±	0.47	30.49	±	1.29	21.58	±	0.60	30.15 #	±	0.48

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

# $P<0.05$; Significant difference between DMSO treatment in Cerebarl ischemia group vs. Sham operation group.

Table 4 : Effects of piperine treatment (5 days) on performance of Cerebral ischemia mice in spatial memory task. → Attenuation of learning and memory impairment.

	Control			P 0.1 mg/kg			P 0.5 mg/kg			P 1 mg/kg			P 5 mg/kg		
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Day0	32.28	±	1.82	33.05	±	2.64	33.40	±	2.62	32.73	±	2.95	32.90	±	2.69
Day1	32.94	±	2.00	19.06 *	±	0.66	22.33 *	±	1.32	25.64 *	±	1.21	29.95 *	±	1.72
Day2	35.45	±	2.23	15.63 *	±	0.85	17.67 *	±	1.04	21.09 *	±	1.17	23.91 *	±	0.82
Day3	28.46	±	3.08	13.97 *	±	0.90	15.96 *	±	0.84	18.82 *	±	0.87	19.43 *	±	1.31
Day4	26.14	±	3.05	13.71 *	±	1.30	15.80 *	±	0.77	17.74 *	±	0.66	18.26 *	±	0.89
Day5	27.74	±	3.33	14.19 *	±	0.84	14.86 *	±	0.87	16.54 *	±	0.83	15.22 *	±	1.00
Mean	30.15	±	0.48	15.31 *	±	0.44	17.32 *	±	0.48	19.97 *	±	0.49	21.35 *	±	0.64

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

* P<0.05; Significant difference between Cerebarl ischemia group treated with Piperine vs. Control (DMSO).

Table 5 : Effects of piperine treatment (5 days) on performance of Sham operation mice in spatial memory task. → Attenuation of learning and memory impairment.

	Control			P 0.1 mg/kg			P 0.5 mg/kg			P 1 mg/kg			P 5 mg/kg		
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Day0	31.54	±	1.84	31.93	±	1.59	32.38	±	1.99	31.04	±	2.53	31.82	±	1.91
Day1	27.91	±	1.72	17.40 *	±	1.05	19.38 *	±	2.22	24.80	±	1.15	26.41	±	0.79
Day2	24.06	±	1.25	15.08 *	±	0.97	17.36 *	±	0.94	20.13	±	1.40	20.04	±	0.53
Day3	19.92	±	1.17	12.93 *	±	0.67	15.65 *	±	0.86	17.72	±	1.50	17.35	±	0.87
Day4	17.31	±	0.80	14.04 *	±	0.94	15.51 *	±	0.96	17.24	±	0.83	16.98	±	0.64
Day5	18.70	±	1.41	12.81 *	±	0.80	14.86 *	±	1.12	16.04	±	0.82	16.88	±	0.70
Mean	21.58	±	0.60	14.45 *	±	0.42	16.55 *	±	0.60	19.19	±	0.57	19.53	±	0.41

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

P<0.05; Significant difference between Sham operation group treated with Piperine vs. Control (DMSO).

Table 6 : The performance of mice in spatial memory task of scopolamine 0.5 and 1 mg/kg administration.

	NSS			Scopolamine 0.5 mg/kg			Scopolamine 1 mg/kg		
	Mean		SEM	Mean		SEM	Mean		SEM
Day0	30.08	±	1.12	34.51	±	1.72	36.17	±	1.99
Day1	23.25	±	3.11	38.78 *	±	3.41	42.27 *	±	3.99
Day2	16.71	±	1.79	37.45 *	±	3.03	42.51 *	±	4.52
Day3	13.57	±	1.52	34.48 *	±	3.36	38.02 *	±	3.89
Day4	11.22	±	0.93	30.02 *	±	3.12	33.77 *	±	4.24
Day5	12.81	±	0.80	27.90 *	±	2.26	30.61 *	±	2.24
Mean	15.51	±	1.63	33.73 *	±	3.04	37.44 *	±	3.78

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

* P<0.05; Significant difference between Scopolamine administration group vs. NSS group.

Table 7 : Effect of piperine on scopolamine 0.5 mg/kg administration-induced memory impairment in mice.

	Scopolamine 0.5 mg/kg											
	Control			Piperine 0.1 mg/kg			Piperine 0.5 mg/kg			Piperine 1 mg/kg			Piperine 5 mg/kg		
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Day0	34.51	±	1.72	33.69	±	1.62	30.95	±	1.44	32.18	±	2.68	32.61	±	1.67
Day1	38.78	±	3.41	36.50	±	3.57	35.88	±	3.82	37.10	±	4.11	40.13	±	4.07
Day2	37.45	±	3.03	34.40	±	4.14	35.39	±	3.53	36.76	±	4.05	37.51	±	3.40
Day3	34.48	±	3.36	31.18	±	2.17	30.96	±	3.95	33.20	±	3.58	33.04	±	3.05
Day4	30.02	±	3.12	27.30	±	3.81	28.40	±	3.23	28.50	±	3.36	29.59	±	3.78
Day5	27.90	±	2.26	23.90	±	2.90	23.42	±	2.16	24.67	±	2.35	26.08	±	3.37
Mean	33.73	±	3.04	30.66	±	3.32	30.81	±	3.34	32.05	±	3.49	33.27	±	3.53

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

Table 8 : Effect of piperine on scopolamine 1 mg/kg administration-induced memory impairment in mice.

	Scopolamine 0.1 mg/kg											
	Control			Piperine 0.1 mg/kg			Piperine 0.5 mg/kg			Piperine 1 mg/kg			Piperine 5 mg/kg		
	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM	Mean		SEM
Day0	36.17	±	1.99	31.78	±	3.63	34.38	±	2.46	33.46	±	1.83	34.64	±	2.47
Day1	42.27	±	3.99	39.45	±	4.18	37.50	±	3.65	40.30	±	3.86	43.80	±	4.11
Day2	42.51	±	4.52	37.77	±	4.52	38.73	±	4.33	39.90	±	2.99	41.60	±	2.25
Day3	38.02	±	3.89	35.40	±	4.09	34.80	±	3.33	37.30	±	2.28	36.50	±	3.11
Day4	33.77	±	4.24	29.41	±	4.04	29.90	±	2.56	30.70	±	4.36	31.39	±	3.39
Day5	30.61	±	2.24	26.03	±	2.46	26.00	±	1.99	27.90	±	2.92	29.70	±	2.55
Mean	37.44	±	3.78	33.61	±	3.86	33.39	±	3.17	35.22	±	3.28	36.60	±	3.08

Unit expressed the escape latency as second

Data shown were mean ± SEM (n=8)

Table 9 : Effect of Piperine administration on locomotor activity.

	Control			Piperine 0.1 mg/kg			Piperine 0.5 mg/kg			Piperine 1 mg/kg			Piperine 5 mg/kg		
	Counts		SEM	Counts		SEM	Counts		SEM	Counts		SEM	Counts		SEM
5 min.	76.38	±	16.25	67.00	±	19.55	74.00	±	14.12	59.13	±	13.17	74.13	±	22.31
10 min.	42.38	±	18.72	35.25	±	7.42	32.50	±	5.73	28.38	±	13.39	48.75	±	15.82
15 min.	22.25	±	6.14	42.50	±	11.59	41.75	±	9.14	27.13	±	11.12	27.25	±	7.11
20 min.	50.38	±	17.92	27.75	±	4.16	37.50	±	9.32	23.88	±	5.17	34.25	±	10.57
25 min.	27.75	±	8.72	35.63	±	8.43	28.75	±	5.50	17.75	±	5.81	23.00	±	5.34
30 min.	21.88	±	4.89	37.13	±	11.57	23.00	±	6.79	28.63	±	11.24	16.88	±	4.98
35 min.	19.13	±	5.58	20.38	±	4.97	13.75	±	3.87	12.38	±	5.38	26.50	±	9.63
40 min.	12.00	±	2.68	20.25	±	5.19	20.00	±	7.85	20.63	±	6.16	28.75	±	12.20
45 min.	8.88	±	3.42	19.25	±	8.90	18.75	±	7.34	18.63	±	7.17	20.88	±	8.26
50 min.	12.88	±	6.05	12.75	±	4.75	15.38	±	8.16	20.50	±	6.81	12.13	±	3.81
55 min.	22.75	±	14.88	29.38	±	22.17	13.75	±	4.19	12.63	±	4.17	12.63	±	3.72
60 min.	49.00	±	18.87	47.38	±	16.16	34.63	±	15.53	21.50	±	7.76	16.63	±	5.53
Total	365.63	±	24.75	394.63	±	29.13	353.75	±	25.11	291.13	±	21.46	341.75	±	24.76

Unit expressed the counts of motor activity (Gross movements)

Data shown were mean ± SEM (n=8)

Table10 : Effect of NSS on Cerebral ischemia, Sham operation and Naive condition in the measurement of Thiobarbituric acid (TBARs Assay).

	NSS		
	Normal	SHAM	2VO
1	22.83	30.31	42.17
2	25.48	29.09	34.15
3	18.89	24.77	34.82
4	20.75	26.75	35.44
Mean	21.99	27.73 *	36.64 *#
SEM	1.41	1.23	1.86

Unit expressed the nmol/ g tissue of standard MDA

Data shown were mean ± SEM (n=4)

* P<0.05; Significant difference from value in normal mice group.

# P<0.05; Significant difference from value in Sham operated condition.

Table 11 : Effect of NSS and DMSO administration on Cerebral ischemia and Sham operation in the measurement of Thiobarbituric acid (TBARs Assay).

	NSS		DMSO	
	SHAM	2VO	SHAM	2VO
1	30.31	42.17	28.77	37.96
2	29.09	34.15	25.14	42.30
3	24.77	34.82	24.79	37.05
4	26.75	35.44	27.69	33.53
Mean	27.73	36.64	26.60	37.71
SEM	1.23	1.86	0.97	1.80

Unit expressed the nmol/ g tissue of standard MDA

Data shown were mean ± SEM (n=4)

Table 12 : The effect of Piperine treatment on 2VO- and Sham-operation in the measurement of thiobarbituric acid (TBARs Assay).

	Control		Piperine 0.1 mg/kg		Piperine 0.5 mg/kg		Piperine 1 mg/kg		Piperine 5 mg/kg	
	SHAM	2VO	SHAM	2VO	SHAM	2VO	SHAM	2VO	SHAM	2VO
1	28.77	37.96	17.94	26.83	26.44	30.22	22.35	35.04	33.85	35.78
2	25.14	42.30	22.53	20.97	23.60	20.16	24.80	24.71	24.94	31.85
3	24.79	37.05	21.58	20.62	22.42	24.94	25.88	28.59	27.61	32.49
4	27.69	33.53	19.12	21.07	17.78	23.70	22.35	27.36	28.39	32.46
Mean	26.60	37.71	20.29 #	22.37 *	22.56	24.75 *	23.85	28.93 *	28.70	33.14
SEM	0.97	1.80	1.06	1.49	1.80	2.08	0.89	2.19	1.87	0.89

Unit expressed the nmol/ g tissue of standard MDA

Data shown were mean ± SEM (n=4)

*Significantly different from value in control (DMSO) group of cerebral ischemia condition.

#Significantly different from value in control (DMSO) group of Sham operated condition.

Curriculum Vitae

Mr. Surachai Pensirinapa was born in February 5, 1977, in Bangkok, Thailand. He graduated with a Bachelor of Science in Pharmacy in 1998 from the Faculty of Pharmaceutical Sciences, Silpakorn University, Thailand. After graduation, he worked as a pharmacist at Boots Retail company for a year before enrollment into the Master of Science in Pharmacy course in Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.