

# CHAPTER IV

## RESULTS

### 1. Behavioral Test

#### 1.1 Effects of asiaticoside on locomotor activity of $\beta$ -amyloid (25-35) injected mice.

The locomotor activity of mice on day 7 after  $A\beta_{25-35}$  injection measured in 6 treatment groups is shown in Figure 6.  $A\beta_{25-35}$  injection with or without daily administration of asiaticoside at all test doses exerted no significant effect on the animal locomotion.

#### 1.2 Effects of asiaticoside on spontaneous alternation behavior in Y-maze task of $\beta$ -amyloid (25-35) injected mice.

The percent alternation in the Y-maze test on day 8 after  $A\beta_{25-35}$  injection in 6 groups of mice is shown in Figure 7(A). The percent alternation of mice with  $A\beta_{25-35}$  injection was significantly lower than control mice. Daily administration of asiaticoside at 5, 10 and 25 mg/kg/day effectively prevented  $A\beta_{25-35}$ -induced memory impairment while daily administration at 50 mg/kg/day failed to improve this memory deficit.

Total number of arm entries in the same Y-maze test was showed in Figure 7(B).  $A\beta_{25-35}$  injection with or without daily administration of asiaticoside at all test doses exerted no significant effect on exploratory motor behavior.

#### 1.3 Effects of asiaticoside on cognitive performance in water-maze task of $\beta$ -amyloid (25-35) injected mice.

##### *Reference memory*

Escape latencies (the time taken to escape onto the hidden platform) in daily and overall training of the water maze task during day 9–13 after  $A\beta_{25-35}$  injection in 6 groups of mice are shown in Figure 8(A, B). The daily and overall escape latency of  $A\beta_{25-35}$ -injected mice was significantly longer than that of control mice. Daily

administration of asiaticoside at 5, 10 and 25 mg/kg/day markedly attenuated A $\beta$ <sub>25-35</sub>-induced spatial memory impairment while daily administration at 50 mg/kg/day was less effective.

The percent of time spent in the platform quadrant (probe trial) was shown in Figure 8(C). The percent of time spent in the platform quadrant of A $\beta$ <sub>25-35</sub>-injected mice was significantly shorter than that of control mice. Daily administration of asiaticoside at 5, 10 and 25 mg/kg/day significantly recovered A $\beta$ <sub>25-35</sub>-induced impairment in spatial memory retention while daily administration at 50 mg/kg/day was ineffective.

#### *Working memory*

Mean escape latencies in each training day of the water maze task during day 14–16 after A $\beta$ <sub>25-35</sub> injection in 6 groups of mice are shown in Figure 9. A $\beta$ <sub>25-35</sub> injection with or without daily administration of asiaticoside at all test doses exerted no significant effect on the escape latency in every training day.

#### 1.4 Effects of asiaticoside on performance in multiple-trial passive avoidance task of $\beta$ -amyloid (25-35) injected mice.

Mean step-through latencies at one day after multiple training trial (the test was done during day 17–18 after A $\beta$ <sub>25-35</sub> injection) in 6 groups of mice are shown in Figure 10. A $\beta$ <sub>25-35</sub> injection with or without daily administration of asiaticoside at all test doses exerted no significant effect on animal memory retention. However, there seemed to be a trend of beneficial effect of asiaticoside on memory retention in A $\beta$ <sub>25-35</sub> injected mice.

## **2. Chemical Test**

#### 2.1 Effects of asiaticoside on brain protein contents in $\beta$ -amyloid (25-35) injected mice.

The protein contents in the brains of mice after completing all behavioral tests in 6 treatment groups are shown in Figure 11. There were no significant changes in cerebral protein contents in any groups of animals.

## 2.2 Effects of asiaticoside on levels of brain lipid peroxidation in $\beta$ -amyloid (25-35) injected mice.

Levels of brain lipid peroxidation expressed in term of MDA equivalence after completing all behavioral tests in 6 treatment groups are shown in Figure 12. In mice with  $A\beta_{25-35}$  injection, brain lipid peroxidation increased up to approximately 160% of control mice. Daily administration of asiaticoside at 5, 10, 25 and 50 mg/kg/day effectively prevented  $A\beta_{25-35}$ -induced brain lipid peroxidation.

## 2.3 Effects of asiaticoside on total brain GSH contents in $\beta$ -amyloid (25-35) injected mice.

The percent alternation of mice with  $A\beta_{25-35}$  injection was significantly lower than control mice. Daily administration of asiaticoside at 5, 10 and 25 mg/kg/day effectively prevented  $A\beta_{25-35}$ -induced memory impairment while daily administration at 50 mg/kg/day failed to improve this memory deficit.

Total GSH contents in mouse brains after completing all behavioral tests in 6 treatment groups are shown in Figure 13. The brain GSH content in  $A\beta_{25-35}$  injected mice was significantly lower than control mice. Daily administration of asiaticoside at 5, 10 and 25 mg/kg/day effectively prevented  $A\beta_{25-35}$ -induced GSH diminution while daily administration at 50 mg/kg/day had no protective effect.

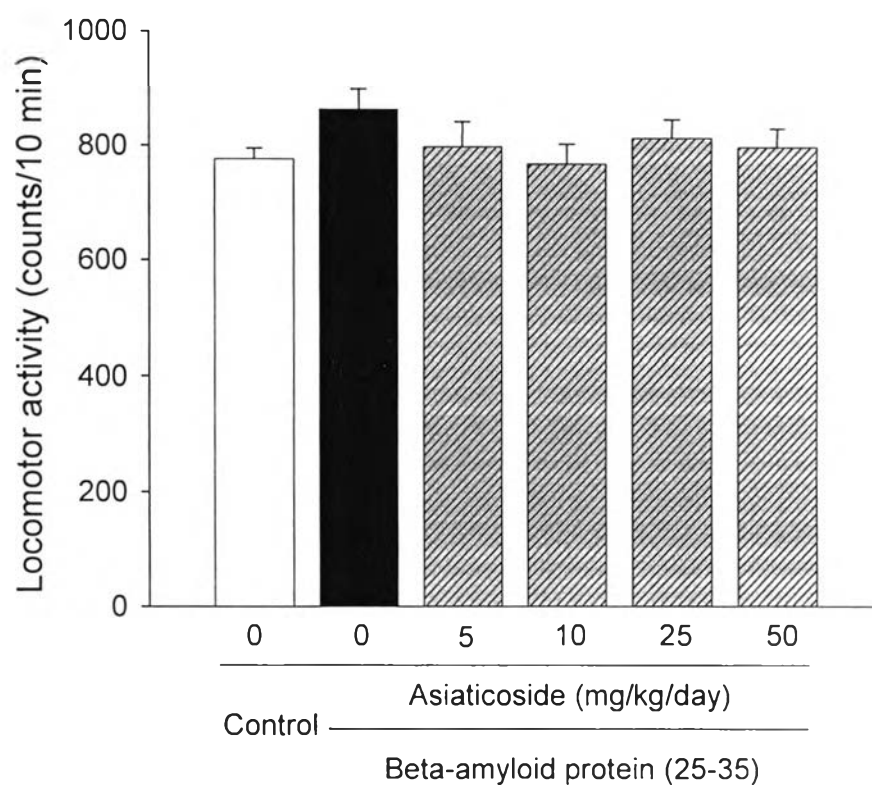


Figure 6 Effects of asiaticoside on locomotor activity of  $\beta$ -amyloid (25-35) injected mice. The locomotor measurement was carried out on day 7 after the start of  $\beta$ -amyloid protein injection. Locomotor activity was measured for 10 min. Columns indicate mean  $\pm$  SEM of values from 8 mice.

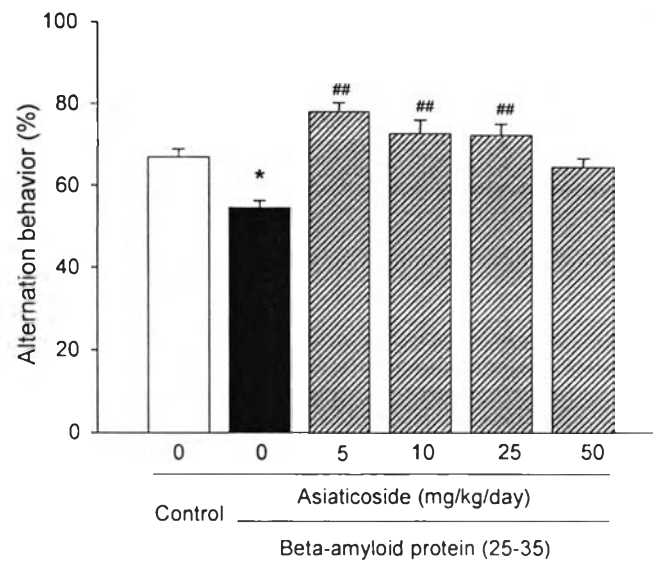
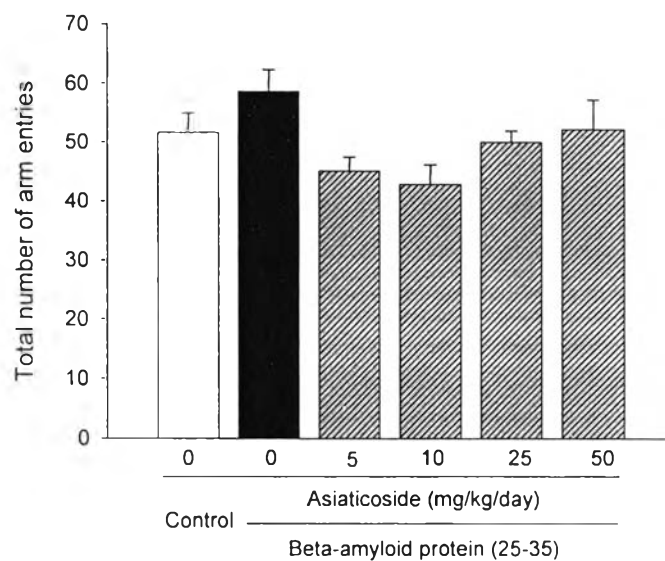
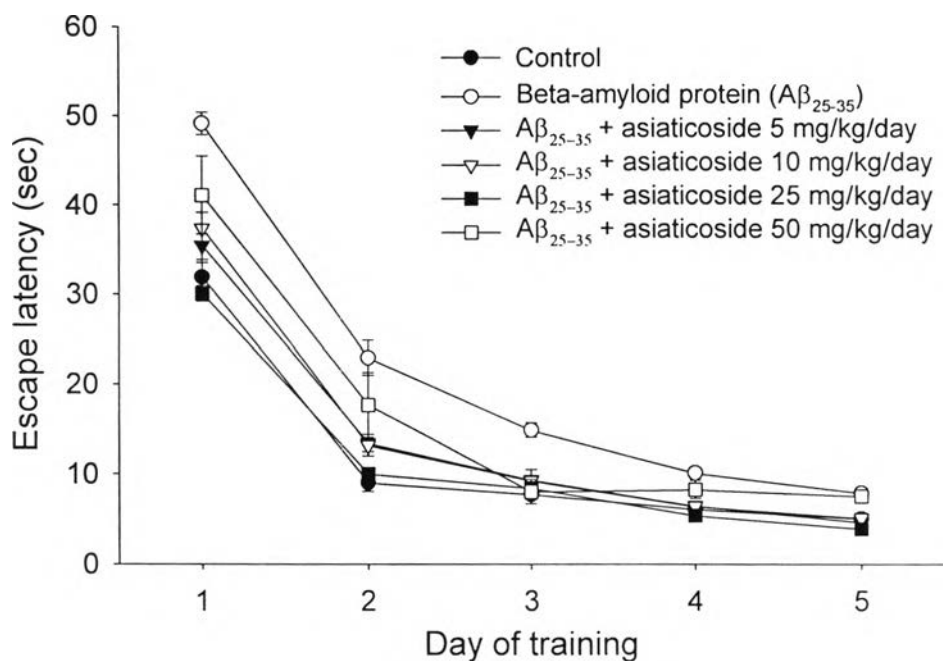
**(A) Percent alternation****(B) Total number of arm entries**

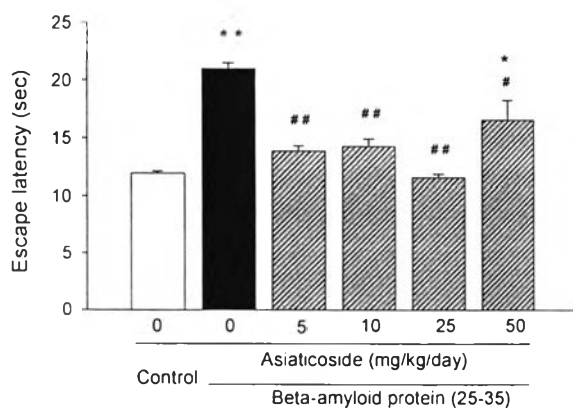
Figure 7 Effects of asiaticoside on spontaneous alternation behavior (A) and the number of arm entries (B) during an 8-min session in the Y-maze task of  $\beta$ -amyloid-injected mice. The task was carried out on day 8 after the start of  $\beta$ -amyloid protein injection. Columns indicate mean  $\pm$  SEM of values from 8 mice.

\*  $P < 0.05$  vs control group, ##  $P < 0.01$  vs  $\beta$ -amyloid-injected group.

## A. Daily platform trials



## B. Overall platform trials



## C. Probe trials

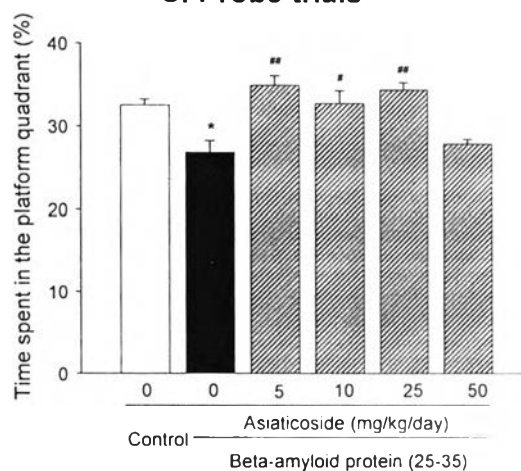
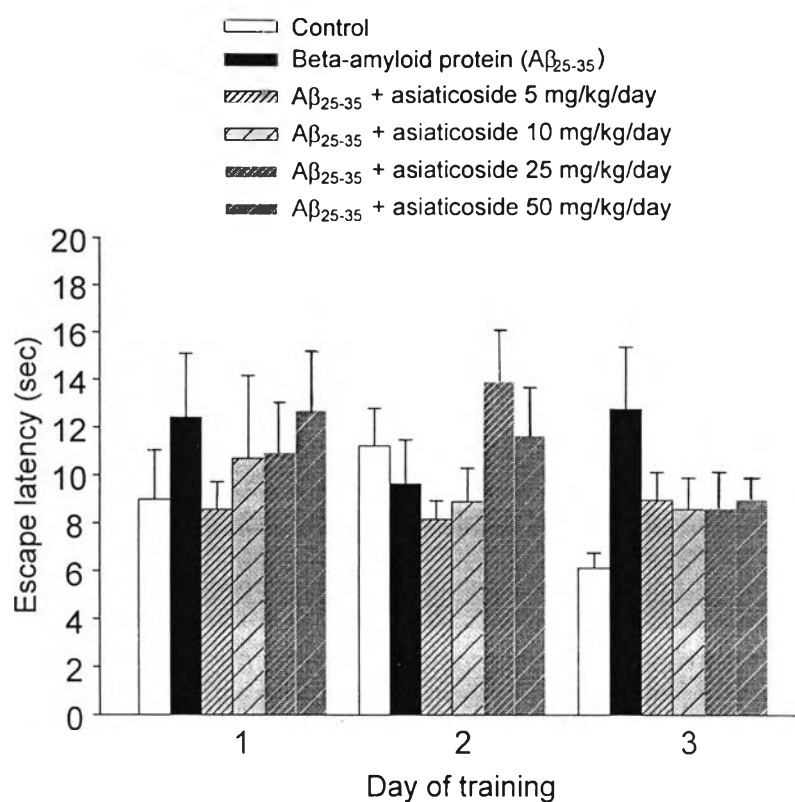


Figure 8 Effects of asiaticoside on reference spatial memory in the Morris water maze task. The hidden-platform trials (A, B) were carried out on day 9-13 after the start of  $\beta$ -amyloid protein injection. The location of platform was fixed throughout 5-day training. The probe trial (C) was carried out on day 13 after the start of  $\beta$ -amyloid protein injection, immediately after the 20<sup>th</sup> hidden-platform trial. Columns indicate mean  $\pm$  SEM of values from 8 mice.

\*  $P < 0.05$  and \*\*  $P < 0.01$  vs control group, #  $P < 0.05$  and ##  $P < 0.01$  vs  $\beta$ -amyloid-injected group.

### A. Daily platform trials



### B. Overall platform trials

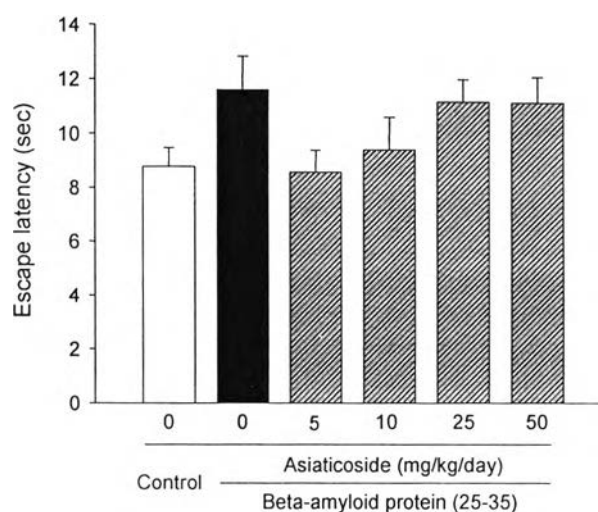


Figure 9 Effects of asiaticoside on working spatial memory in the Morris water maze task. The hidden-platform trials were carried out on day 14-16 after the start of  $\beta$ -amyloid protein injection. The location of platform in the pool was changed every day. Columns indicate mean  $\pm$  SEM of values from 8 mice.

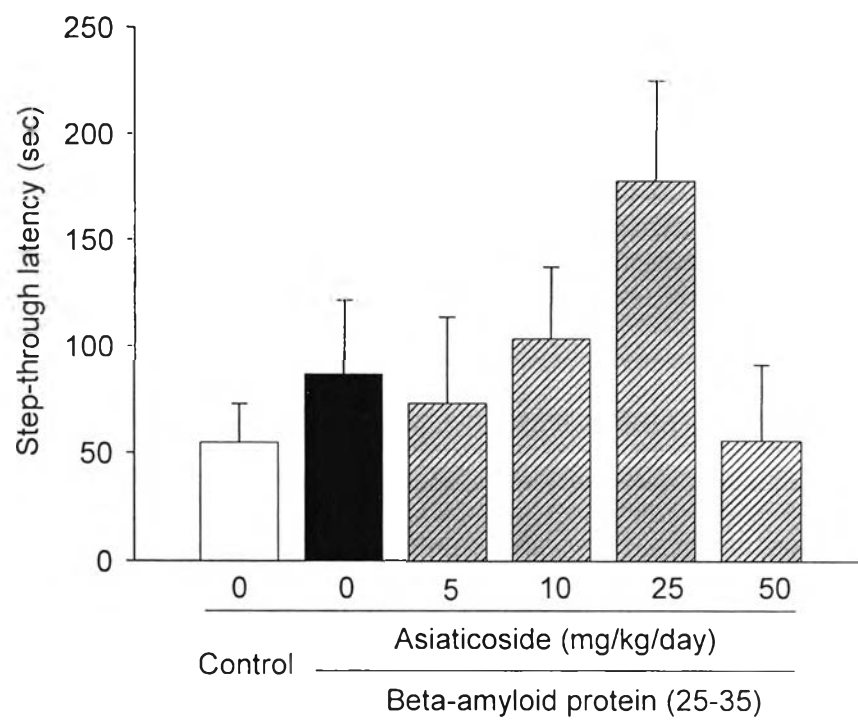


Figure 10 Effects of asiaticoside on the step-through latency in multiple-trial passive avoidance task of  $\beta$ -amyloid injected mice. The task was carried out on day 17-18 after the start of  $\beta$ -amyloid injection. Columns indicate mean  $\pm$  SEM of values from 8 mice.



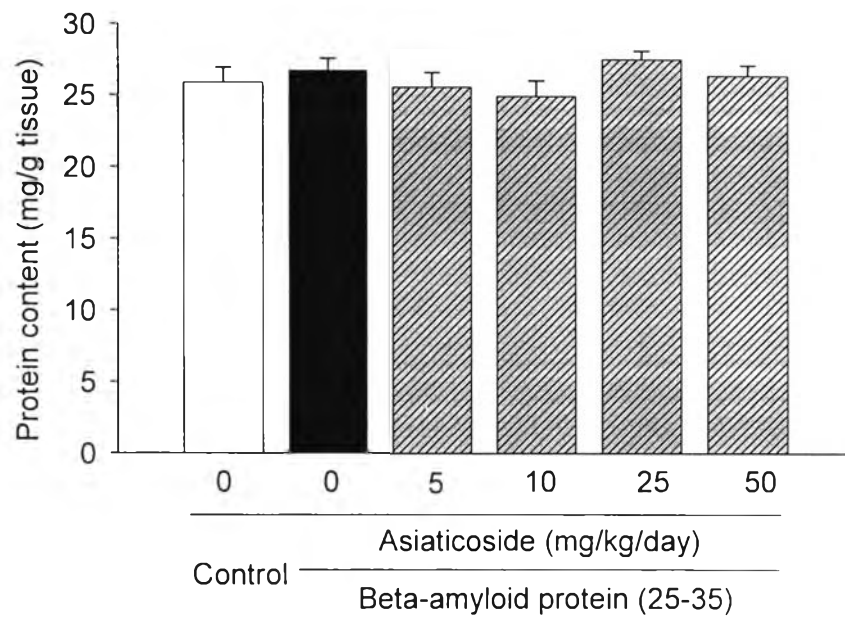


Figure 11 Effects of asiaticoside on brain protein contents in  $\beta$ -amyloid (25-35) injected mice. Protein contents of the cortex samples were measured by Bradford's reagent. Mice were killed on day 19 after the start of  $\beta$ -amyloid injection. Columns indicate mean  $\pm$  SEM of values from 8 mice.

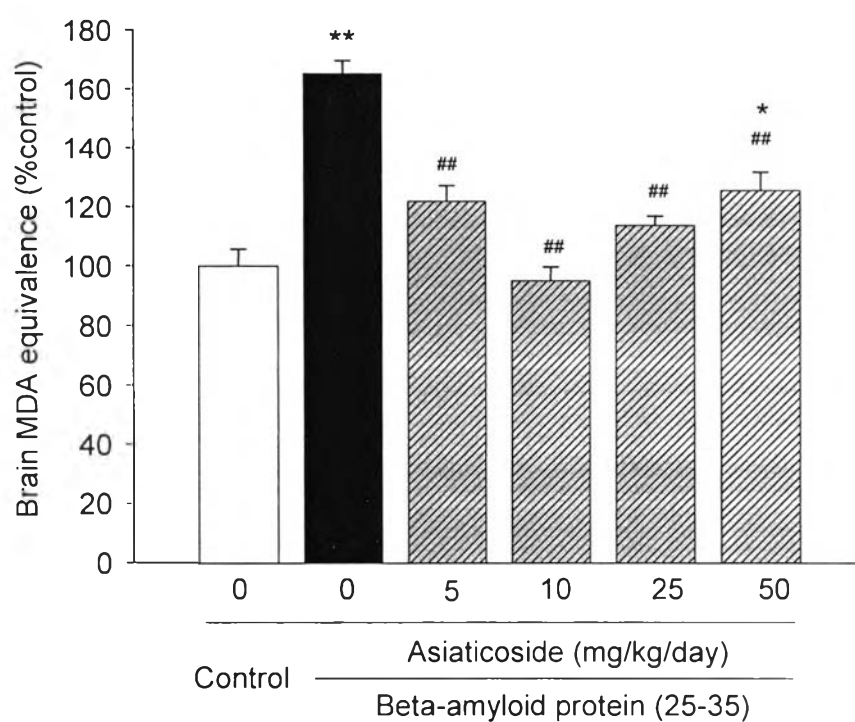


Figure 12 Effects of asiaticoside on levels of brain lipid peroxidation in  $\beta$ -amyloid (25-35) injected mice. Levels of lipid peroxidation in the mouse cerebral cortex after completing all behavioral tests were analyzed by TBARS assay and expressed as MDA equivalence. Columns indicate mean  $\pm$  SEM of values from 8 mice.

\*  $P < 0.05$  and \*\*  $P < 0.01$  vs control group, ##  $P < 0.01$  vs  $\beta$ -amyloid injected group.

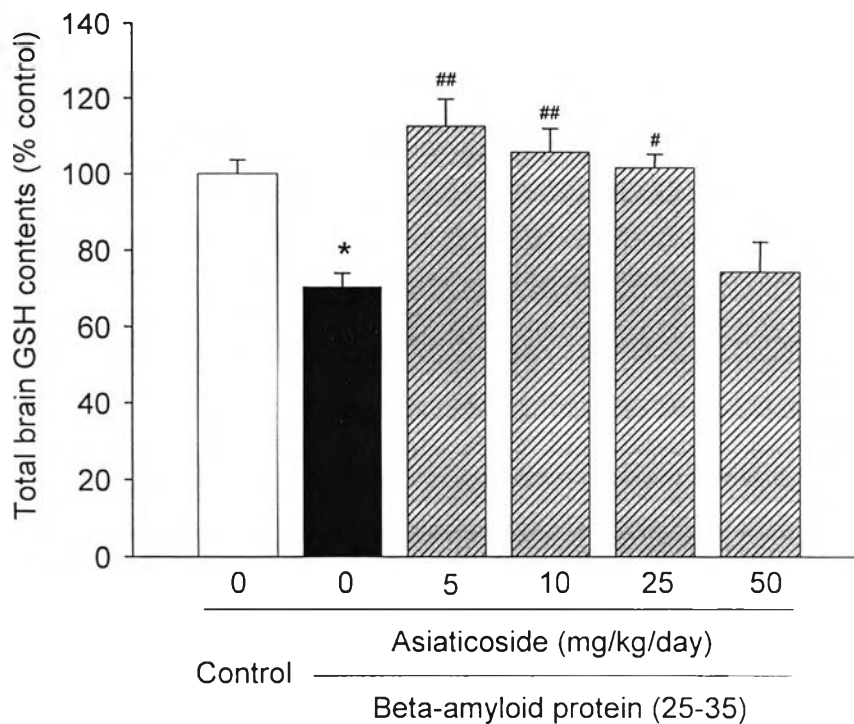


Figure 13 Effects of asiaticoside on total brain GSH contents in  $\beta$ -amyloid (25-35) injected mice. Levels of GSH in the mouse cerebral cortex after completing all behavioral tests were analyzed by Ellman's reagent. Columns indicate mean  $\pm$  SEM of values from 8 mice.

\*  $P < 0.05$  vs control group, #  $P < 0.05$  and ##  $P < 0.01$  vs  $\beta$ -amyloid injected group.