# CHAPTER IV

# RESULTS

## 1. Effects of S-nitrosopennicillamine (SNAP) on mitochondrial activity of N1E-115 neuroblastoma cell line.

Upon stimulation with 0.25 to 3 mM of SNAP for 6, 12 and 24 hr, mitochondrial metabolic activity of cultured N1E-115 cells was markedly decreased accordingly to increasing concentrations when compared with control cultures (Fig. 6). After an exposure of N1E-115 cultures to 1 mM of SNAP for 24 hr, mitochondrial metabolic activity of the cultured cells was decreased to nearly 50% of that of controls.

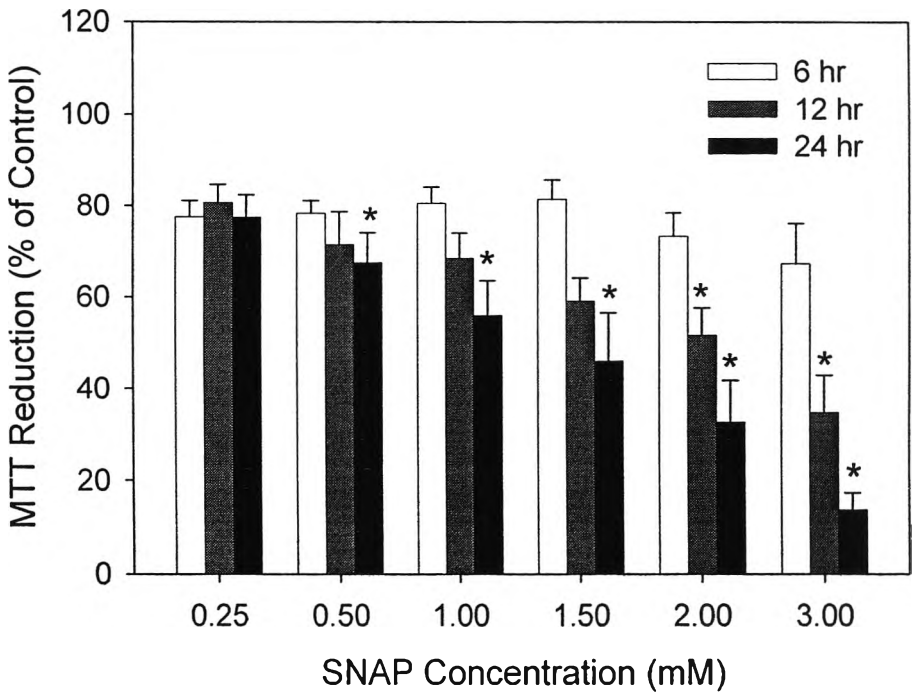


**Fig. 6 Inhibitory effects of SNAP on mitochondrial activity of cultured N1E-115 cell line.**

Cultures were exposed to increasing concentrations of SNAP for 6, 12 and 24 hr before the determination of MTT reduction activity. Values are mean ± S.E.M. (N = 6; with duplicate experiments). * $P<0.05$ compared with control cultures (One-way ANOVA followed by Dunnett's test for multiple comparisons).

**2. Effects of asiaticoside on mitochondrial activity of N1E-115 neuroblastoma cell line.**

Treatment of N1E-115 cultures with a range of asiaticoside concentrations for various time intervals showed that low concentrations of asiaticoside (1-100 μM) had no cytotoxic effects as considered from mitochondrial metabolic activity after 24 and 48 hr of incubation (Fig. 7). In contrast, mitochondrial metabolic activity was markedly decreased at higher concentrations of asiaticoside (200-500 μM) after 24 and 48 hr of incubation.

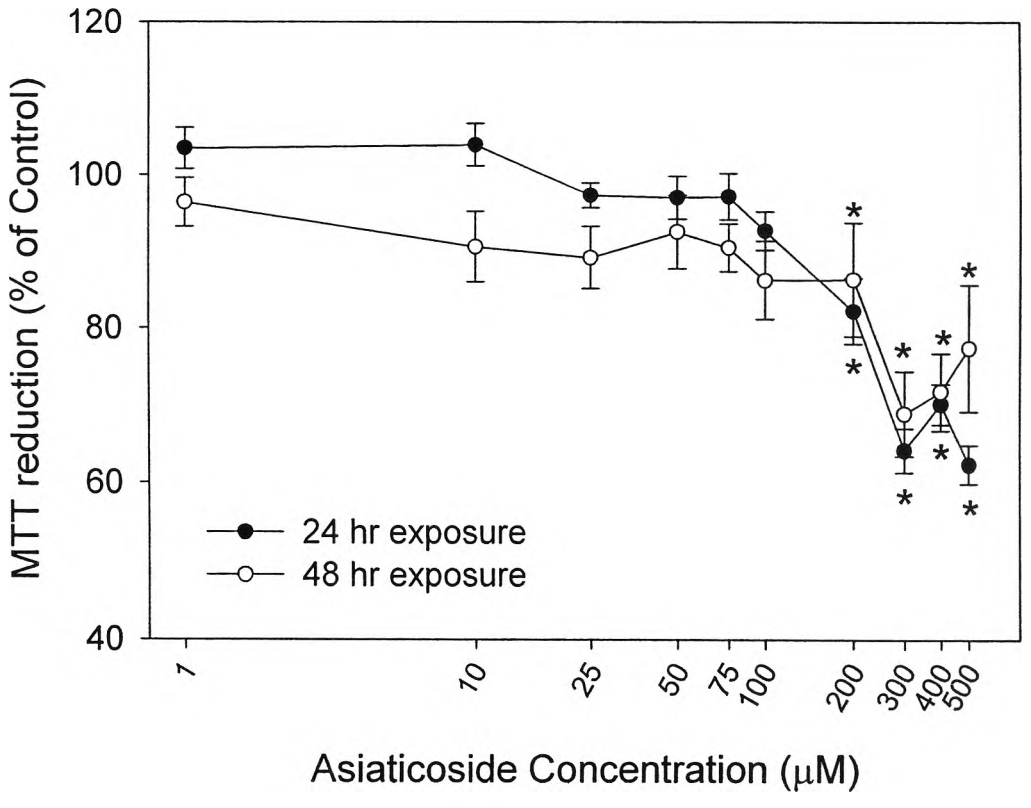


**Fig. 7 Effects of asiaticoside on mitochondrial activity of cultured N1E-115 cell line.**

Cultures were treated with increasing concentrations of asiaticoside. Mitochondrial metabolic activity was estimated 24 and 48 hr later by using the MTT colorimetric assay. Values are mean ± S.E.M. (N = 6; with duplicate experiments). * $P<0.05$ compared with control cultures (One-way ANOVA followed by Dunnett's test for multiple comparisons).

### 3. Effects of *Centella asiatica* extract on mitochondrial activity of N1E-115 neuroblastoma cell line.

Treatment of N1E-115 cultures with a range of *C. asiatica* extract concentrations (1-100 μg/ml) for various time intervals showed that there were no significant changes in mitochondrial metabolic activity after 24 and 48 hr of incubation. (Fig. 8).

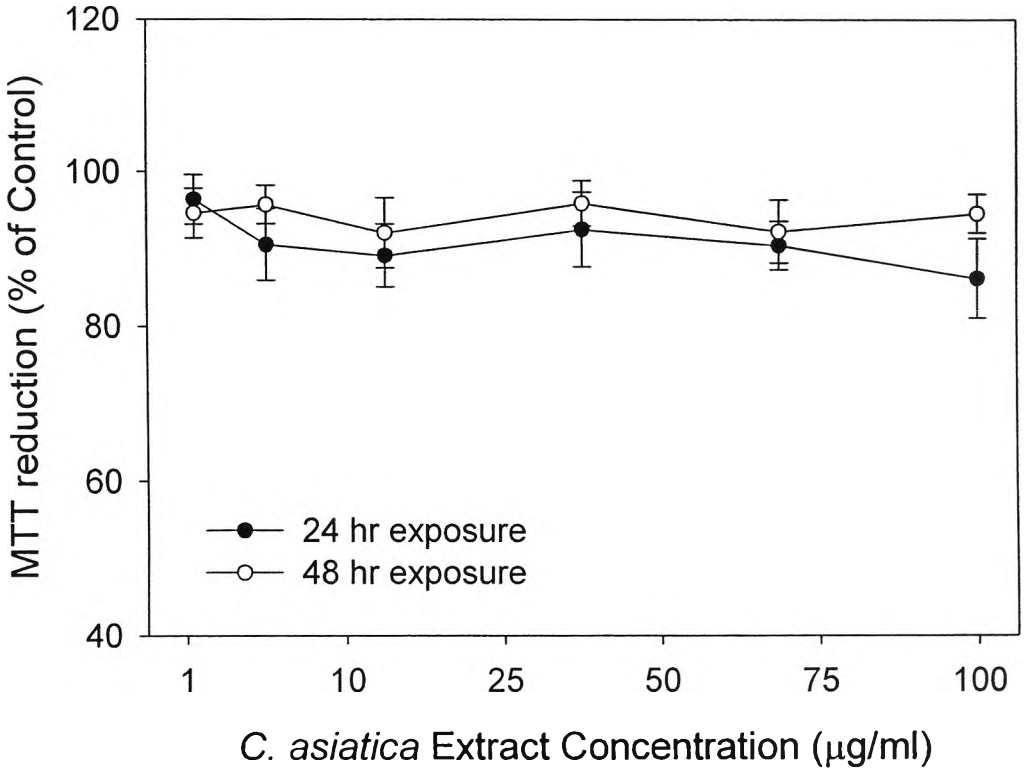


**Fig. 8 Effects of *C. asiatica* extract on mitochondrial activity of cultured N1E-115 cell line.**

Cultures were treated with increasing concentrations of *C. asiatica* extract. Mitochondrial metabolic activity was estimated 24 and 48 hr later by using the MTT colorimetric assay. Values are mean ± S.E.M. (N = 6; with duplicate experiments). Statistical analysis for differences among means was made by one-way ANOVA.

**4. Effects of pre-treatment with asiaticoside on mitochondrial activity of SNAP-exposed N1E-115 neuroblastoma cell line.**

Exposure of N1E-115 cultures to SNAP at a concentration of 1 mM for 24 hr reduced mitochondrial activity nearly 50% in comparison with untreated controls. In this study, pre-incubation with asiaticoside at a range of concentrations of 1-100 μM could not block mitochondrial damage induced by SNAP (Fig. 9).

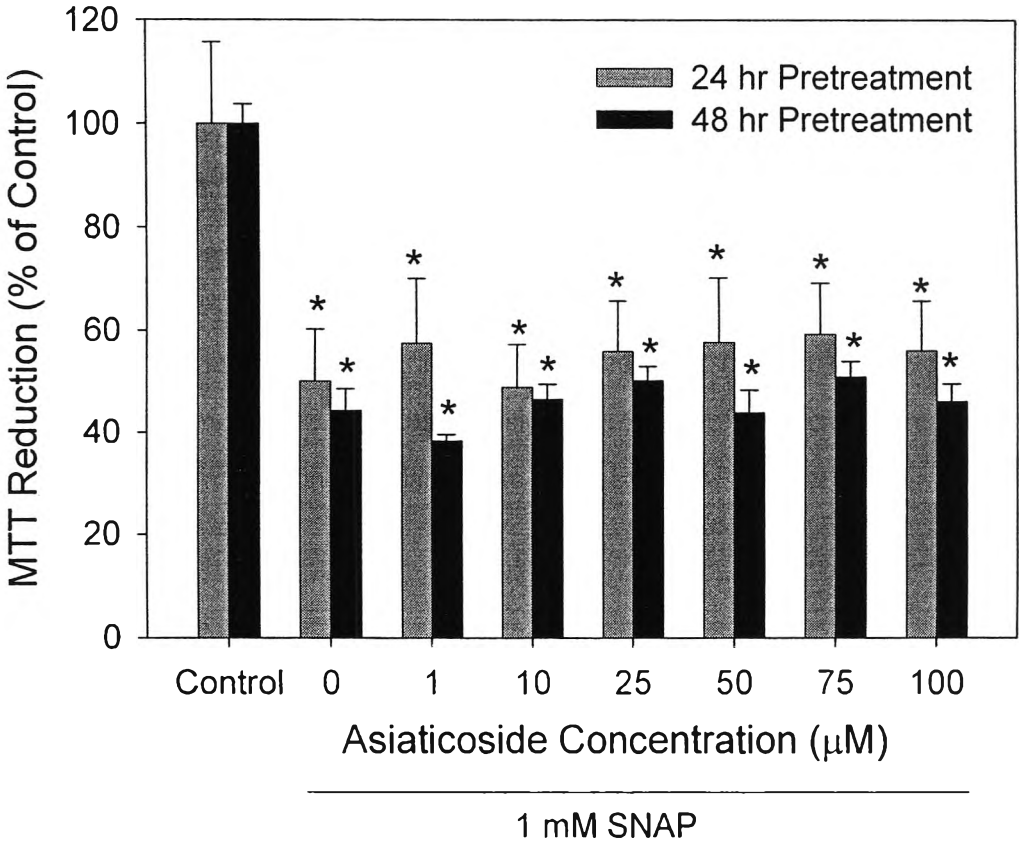


**Fig. 9 Effects of pre-treatment with asiaticoside on mitochodrial activity of cultured N1E-115 cell line exposed to SNAP.**

N1E-115 cultures were incubated with 1-100 μM of asiaticoside for 24 or 48 hr and further incubated with fresh medium containing 1 mM SNAP for 24 hr after which mitochondrial activity was determined. Valued are mean ± S.E.M. (N = 6). * $P<0.05$ compared with control cultures (One-way ANOVA followed by Dunnett's test for multiple comparisons).

**5. Effects of pre-treatment with *C. asiatica* extract on mitochondrial activity of SNAP-exposed N1E-115 neuroblastoma cell line.**

Exposure of N1E-115 cultures to SNAP at a concentration of 1 mM for 24 hr reduced mitochondrial activity nearly 50% in comparison with untreated controls. In this study, pre-incubation with *C. asiatica* extract at a range of concentrations of 1-100 μg/ml could not block mitochondrial damage induced by SNAP (Fig. 10).

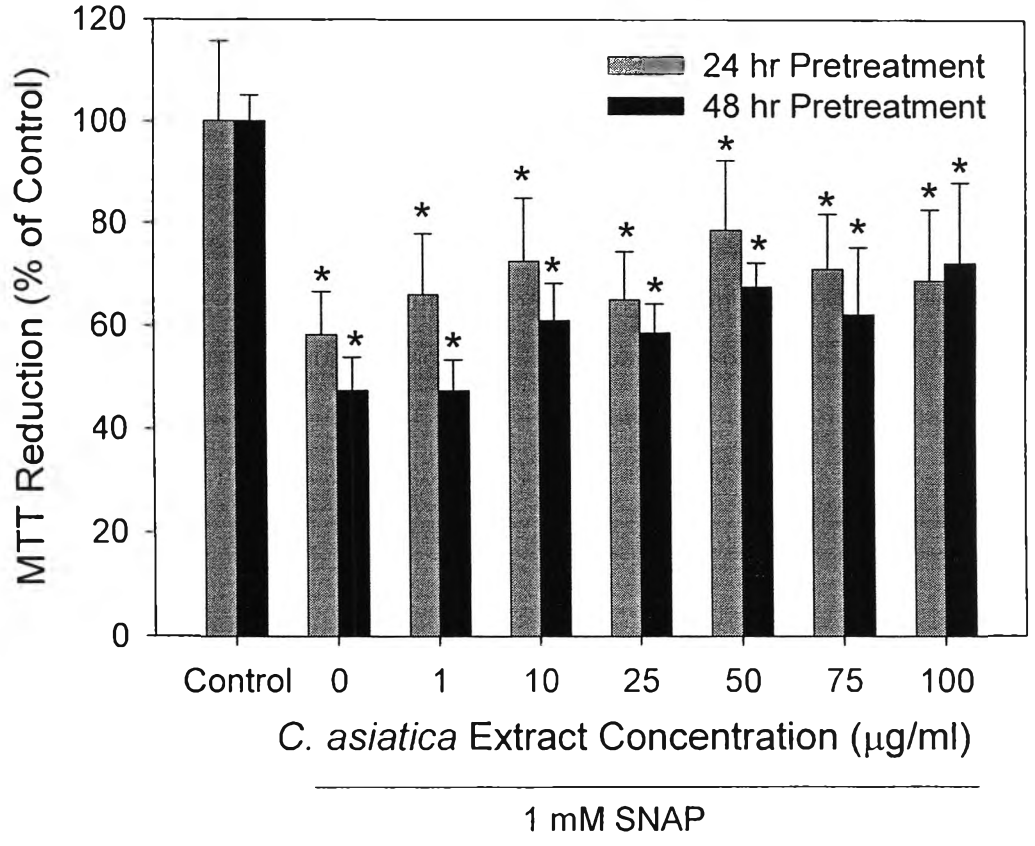


**Fig. 10 Effects of pre-treatment with *C. asiatica* extract on mitochodrial activity of cultured N1E-115 cell line exposed to SNAP.**

N1E-115 cultures were incubated with 1-100 μg/ml of *C. asiatica* extract for 24 or 48 hr and further incubated with fresh medium containing 1 mM SNAP for 24 hr after which mitochondrial activity was determined. Valued are mean ± S.E.M. (N = 6). * $P<0.05$ compared with control cultures (One-way ANOVA followed by Dunnett's test for multiple comparisons).

**6. Effects of co-treatment with asiaticoside on mitochondrial activity of SNAP-exposed N1E-115 neuroblastoma cell line.**

Exposure of N1E-115 cultures to SNAP at a concentration of 1 mM for 24 hr reduced mitochondrial activity to nearly 50% compared in comparison with untreated controls. The mitochondrial damage induced by SNAP could not be blocked by co-incubation with any concentrations of asiaticoside Fig. 11).

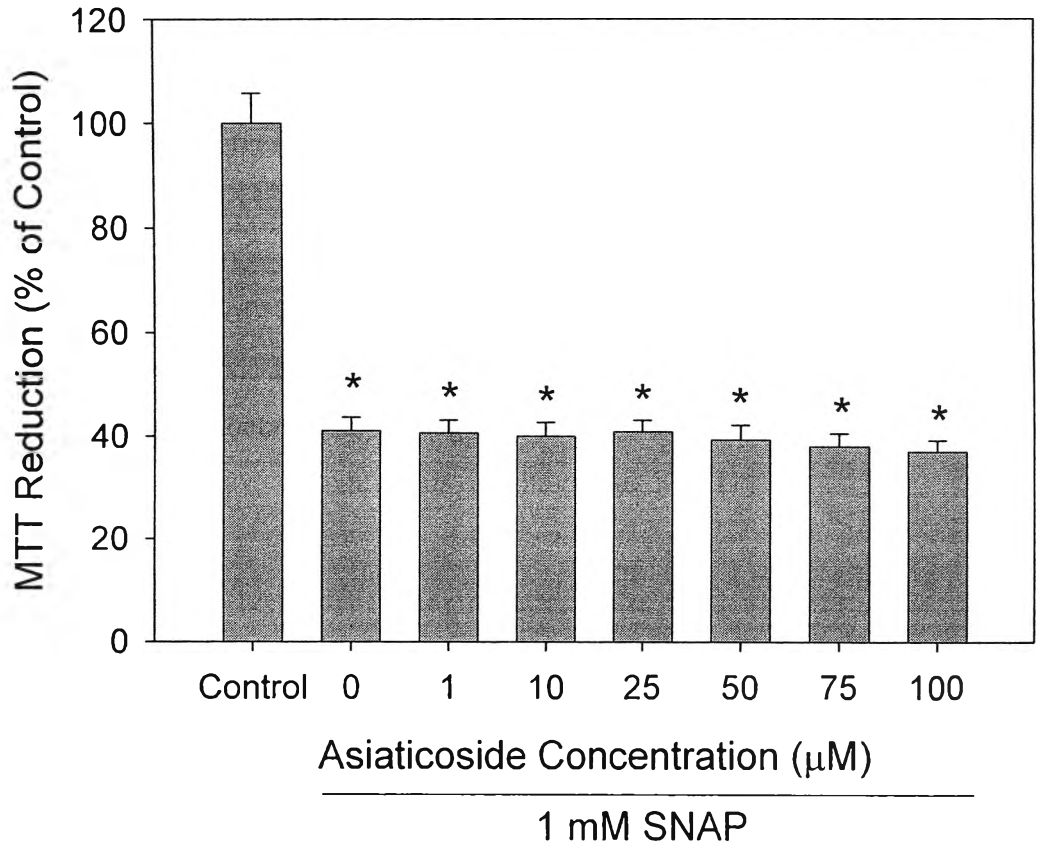


**Fig. 11 Effects of co-treatment with asiaticoside on mitochodrial activity of cultured N1E-115 cell line exposed to SNAP.**

N1E-115 cultures were incubated with 1-100 μM asiaticoside in combination with 1 mM SNAP for 24 hr before determination of cellular MTT reduction. Values are mean ± S.E.M. (N = 6). * $P<0.05$ compared with control cultures (One-way ANOVA followed by Dunnett's test for multiple comparisons).

**7. Effects of co-treatment with *C. asiatica* extract on mitochondrial activity and cell viability of SNAP-exposed N1E-115 neuroblastoma cell line.**

Exposure of N1E-115 cultures to SNAP at a concentration of 1 mM for 24 hr reduced mitochondrail activity to nearly 50% in comparison with untreated controls. However, the mitochondrial damage caused by SNAP was significantly reduced by co-incubation with 25-100 μg/ml of *C. asiatica* extract in a concentration-dependent manner. However, low *C. asiatica* extract concentrations (1-10 μg/ml) did not block mitochondrial damage caused by SNAP (Fig. 12). Under the same culture condition, co-incubation with 50-100 μg/ml of *C. asiatica* extract significantly increased cell viability of SNAP-exposed N1E-115 cell line. In similarity to the effect on mitochondrial activity, low *C. asiatica* extract concentrations (1-25 μg/ml) did not increase cell viability of SNAP-exposed N1E-115. (Fig. 13)

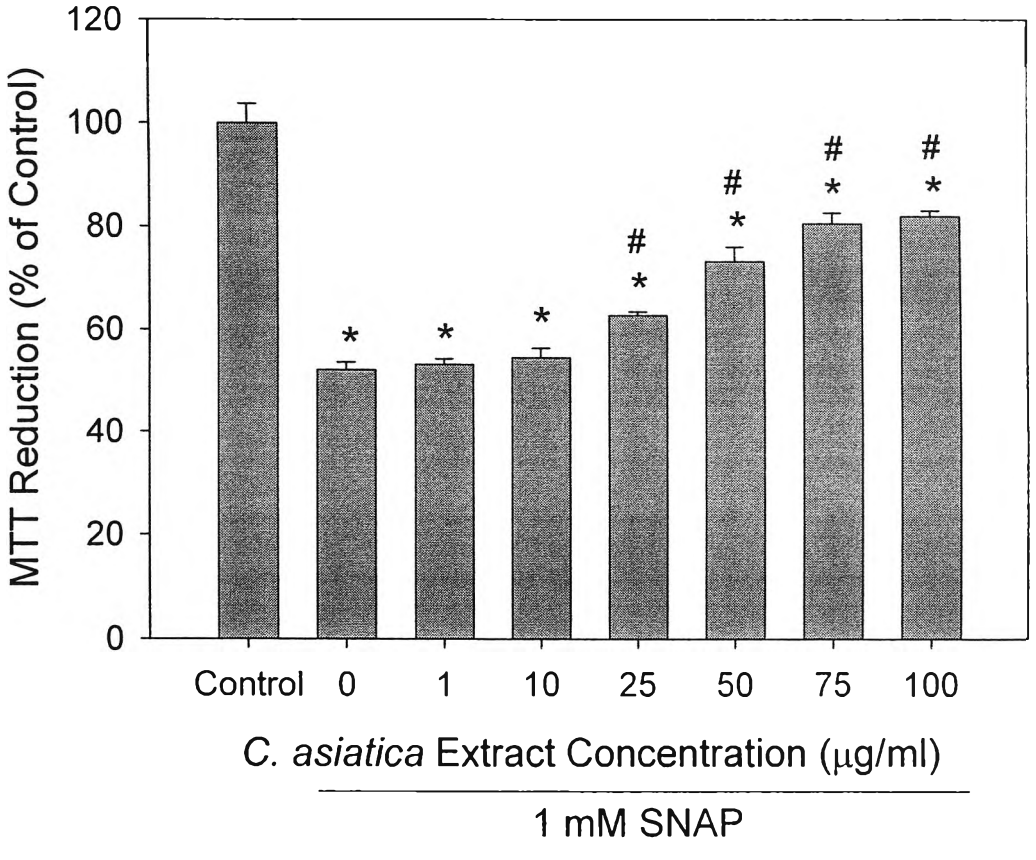


**Fig. 12 Effects of co-treatment with *C. asiatica* extract on mitochondrial activity of cultured N1E-115 cell line exposed to SNAP.**

N1E-115 cultures were incubated with 1-100 μg/ml *C. asiatica* extract in combination with 1 mM SNAP for 24 hr before determination of cellular MTT reduction. Values are mean ± S.E.M. (N = 6). * P<0.05 vs control group, # P<0.05 vs SNAP-exposed group ((One-way ANOVA followed by Dunnett's test for multiple comparisons).

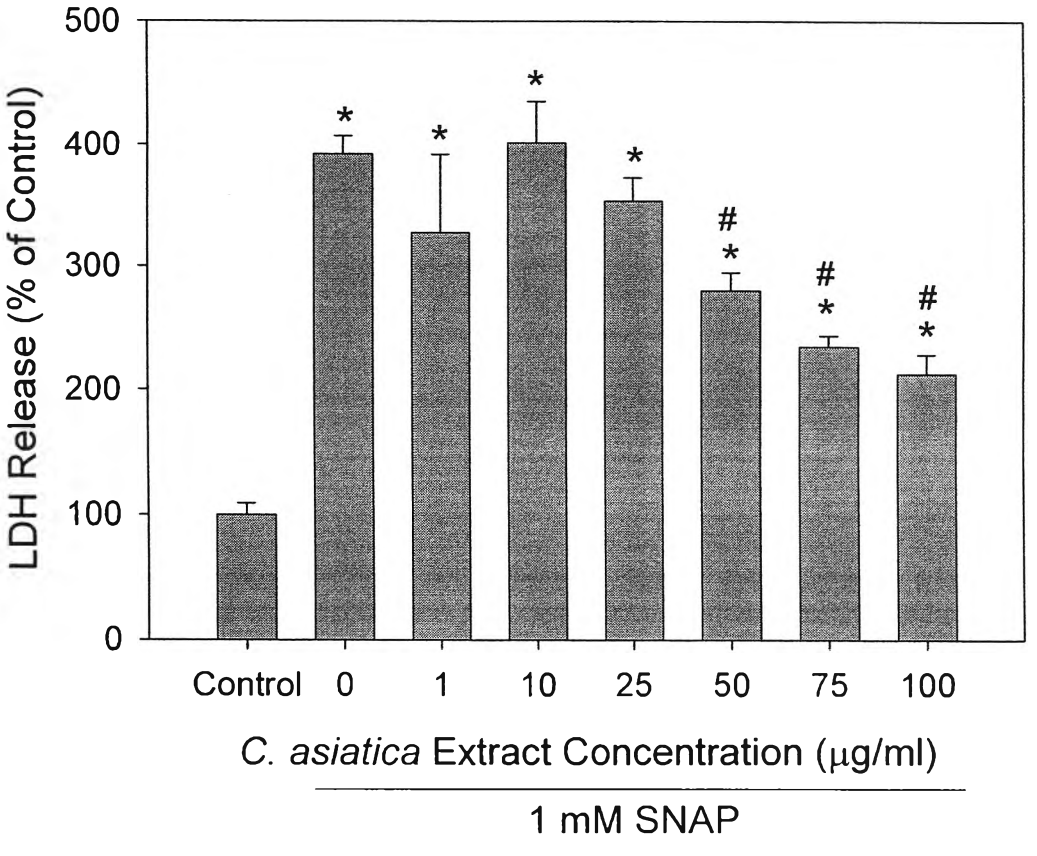


**Fig. 13 Effects of co-treatment with *C. asiatica* extract on cell viability of cultured N1E-115 cell line exposed to SNAP.**

Cultures were incubated with *C. asiatica* extract (1-100 μg/ml) in combination with SNAP (1 mM) for 24 hr before determination of cytoplasmic LDH release. Values are mean ± S.E.M. (N = 6). * P<0.05 vs control group, # P<0.05 vs SNAP-exposed group (One-way ANOVA and Dunnett's t-test).

**8. Effects of co-treatment with *C. asiatica* extract on nitrite accumulation in SNAP-exposed N1E-115 neuroblastoma cell line.**

Co-incubation with 1-100 μg/ml *C. asiatica* extract and SNAP (1 mM) for 24 hr did not change SNAP-induced nitrite accumulation in cultured N1E-115 cells. After 24 hr of incubation with 1 mM SNAP, nitrite content was increased in the medium of N1E-115 cultures. However, *C. asiatica* extract, at all concentrations tested, did not affect the increase of nitrite accumulation in the supernatant of SNAP-exposed N1E-115 cultures (Fig. 14).

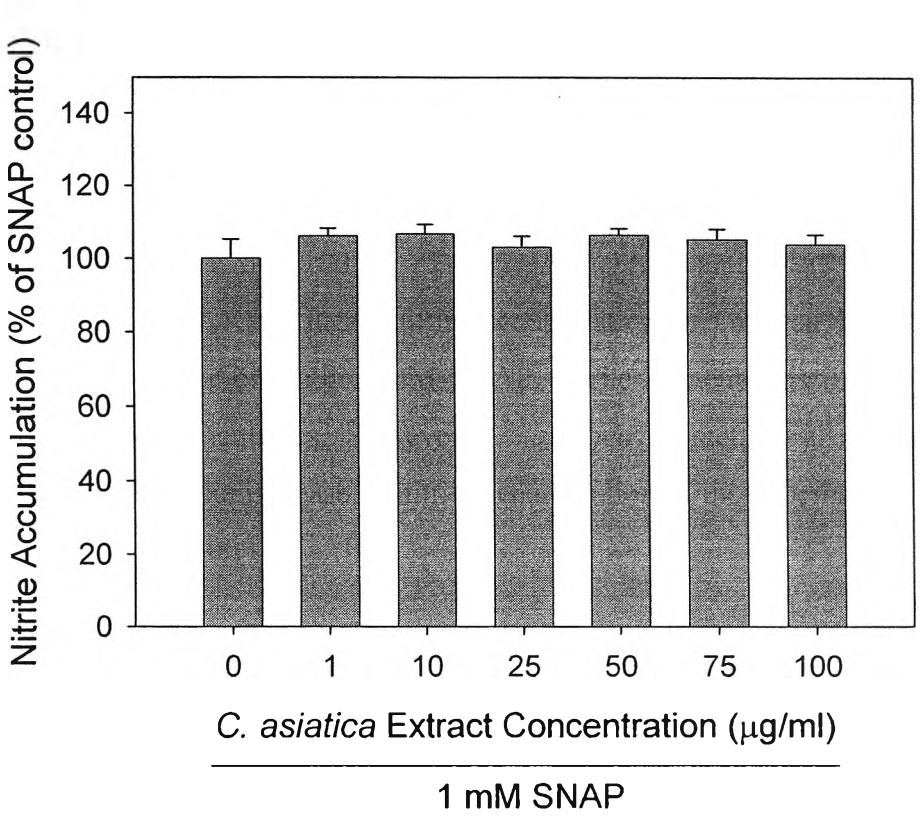


**Fig. 14 Effects of co-treatment with *C. asiatica* extract on nitrite accumulation in cultured N1E-115 cell line exposed to SNAP.**

Cultures were incubated with 1 mM SNAP and *C. asiatica* extract (1-100 μg/ml) for 24 hr after which nitrite accumulation in the medium was determined by Griess reagent. Nitrite accumulation in untreated control cultures was not detectable. Values are mean ± S.E.M. (N = 6). Statistical analysis for differences among means was made by one-way ANOVA.

**9. Effects of co-treatment with *C. asiatica* extract on glutathione levels of SNAP-exposed N1E-115 neuroblastoma cell line.**

Co-treatment with 100 µg/ml of *C. asiatica* extract and 1 mM SNAP for 24 hr slightly reversed SNAP-induced glutathione content diminution in cultured N1E-115 cells. This effect of SNAP was no significantly attenuated when treated N1E-115 with *C. asiatica* extract (Fig. 15).

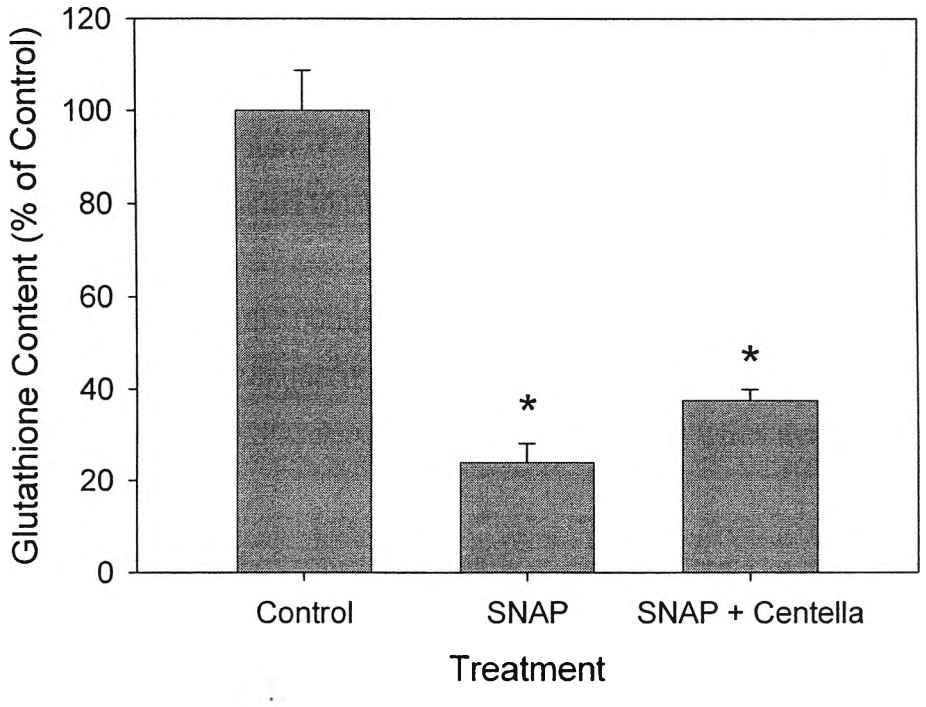


**Fig. 15 Effects of co-treatment with *C. asiatica* extract on glutathione levels of cultured N1E-115 cell line exposed to SNAP.**

Cultures were incubated with 100 µg/ml of *C. asiatica* extract and 1 mM SNAP for 24 hr before glutathione content determination. Values are mean ± S.E.M. (N = 6). ). * P<0.05 vs control group (One-way ANOVA followed by Dunnett's test for multiple comparisons).

**10. Effects of co-treatment with *C. asiatica* extract on levels of lipid peroxidation in SNAP-exposed N1E-115 neuroblastoma cell line.**

Cellular lipid peroxidation was measured with a spectrofluorometric method (TBARS). After co-treatment of cultured N1E-115 cells with 100 μg/ml of *C. asiatica* extract and 1 mM of SNAP for 24 hr, levels of MDA were determined. The result showed that co-treatment with *C. asiatica* extract and SNAP increased MDA levels when compared with SNAP-exposed group. These results suggest that protective effect of *C. asiatica* on SNAP-induced cell injury may not mediate through lowering lipid peroxidation. It may use by other mechanisms which will be discussed in the next chapter (Fig. 16).

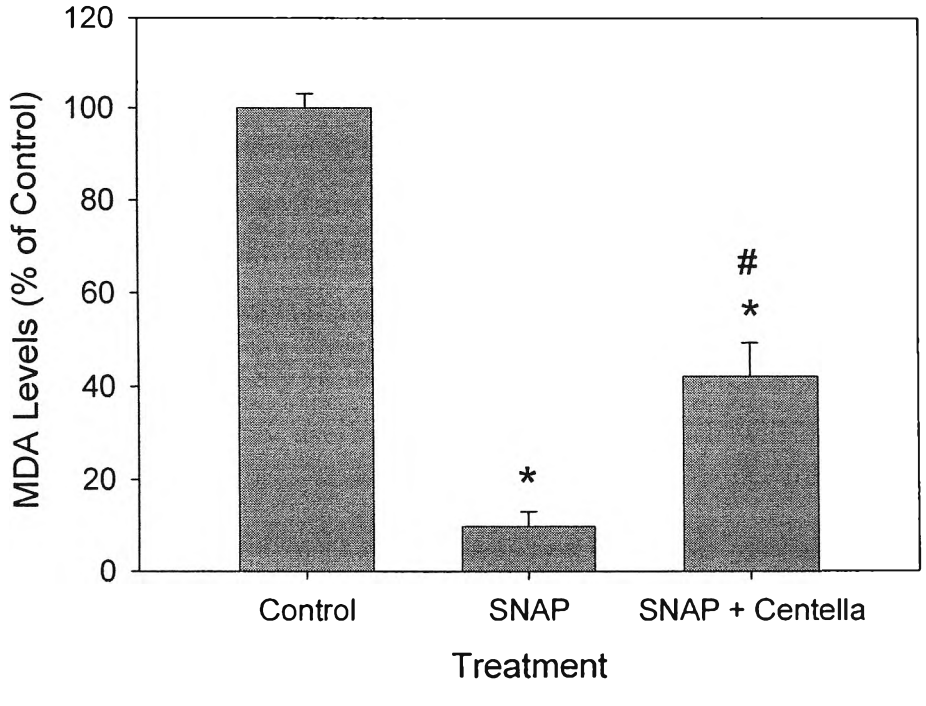


**Fig. 16 Effects of co-treatment with *C. asiatica* extract on level of lipid peroxidation in cultured N1E-115 cell line exposed to SNAP.**

Cultures were incubated with 100 μg/ml of *C. asiatica* extract and 1 mM SNAP for 24 hr before determining levels of lipid peroxidation by TBARS assay. Values are mean ± S.E.M. (N = 6). ). * $P<0.05$ vs control group, # $P<0.05$ vs SNAP-exposed group (One-way ANOVA followed by Dunnett's test for multiple comparisons).