CHAPTER 3

RESULTS

3.1 Effects of Cadmium on Mitochondrial Function

The effects of different concentrations of cadmium on kidney mitochondrial volume were evaluated by measuring the alterations in absorbance of mitochondrial suspension, wherein the decrease in absorbance reflected mitochondrial swelling. The changes in mitochondrial absorbance were displayed as kinetic profile (Figure 3-1A) as well as endpoint at 10 min (Figure 3-1B). The results demonstrated that the absorbance measured from control mitochondria was stable all the way through the end of experiment. In contrast, treated mitochondria with $CdCl_2$ resulted in a rapidly and significantly decreased in the absorbance throughout the measurement period. In addition, the absorbance reduction was shown to be a proportional dependence on the concentration of cadmium.

Figure 3-2 illustrates the mitochondrial production of ROS in response to $CdCl_2$ administration. There was a significant increase in the amount of mitochondrial ROS compared to the control group when $CdCl_2$ was added to the mitochondrial suspension. A stepwise increase in the mitochondrial ROS level was also noticed as cadmium concentration increased.

The effect of cadmium on mitochondrial membrane potential change ($\Delta\Psi m$) was expressed in term of red/green fluorescence intensity ratio, where a decrease in the ratio implied the dissipation of membrane potential and reflected mitochondrial

depolarization. It was found that administration of 10 μM $CdCl_2$ to the mitochondrial suspension caused a slight, albeit not significant, decrease in the red/green fluorescence intensity ratio compared to that observed in the control group (Figure 3-3). However, substantial reduction in $\Delta\Psi m$ was evident when mitochondria were treated with $CdCl_2$ at higher concentrations.

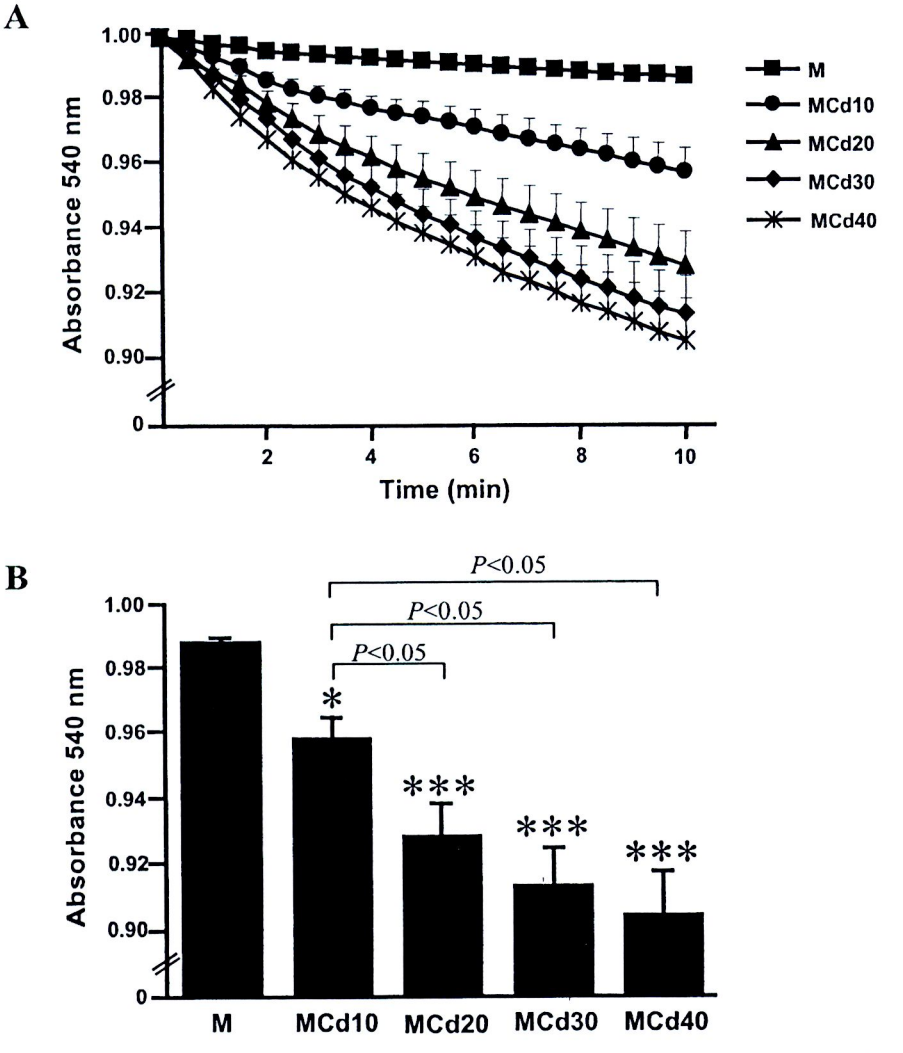

Figure 3-1 Effects of various concentrations of cadmium on mitochondrial swelling (A) kinetics (B) endpoint. Values are means ± SEM from 8 experiments. M, control mitochondria; MCd (10, 20, 30, 40), mitochondria treated with 10, 20, 30, and 40 μM $CdCl_2$, respectively. $*P<0.05$,$**P<0.01$,$***P<0.001$ vs. M group.

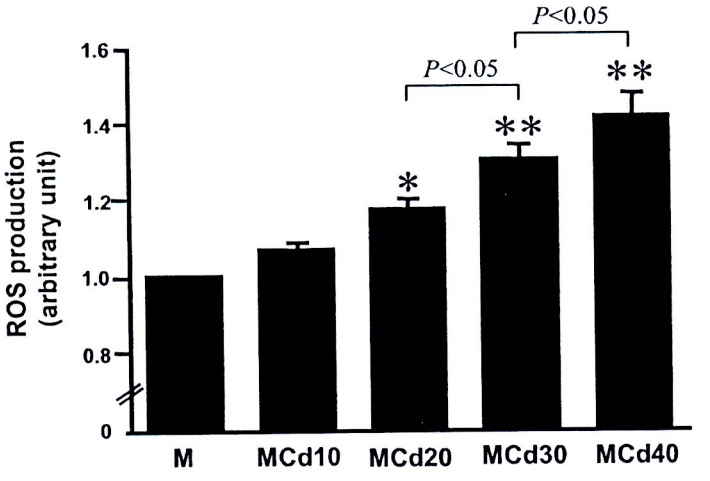

Figure 3-2 Effects of various concentrations of cadmium on mitochondrial ROS production. Values are means ± SEM of 8 experiments. M, control mitochondria; MCd (10, 20, 30, 40), mitochondria treated with 10, 20, 30, and 40 μM $CdCl_2$, respectively. *P<0.01, **P<0.001 vs. M group.

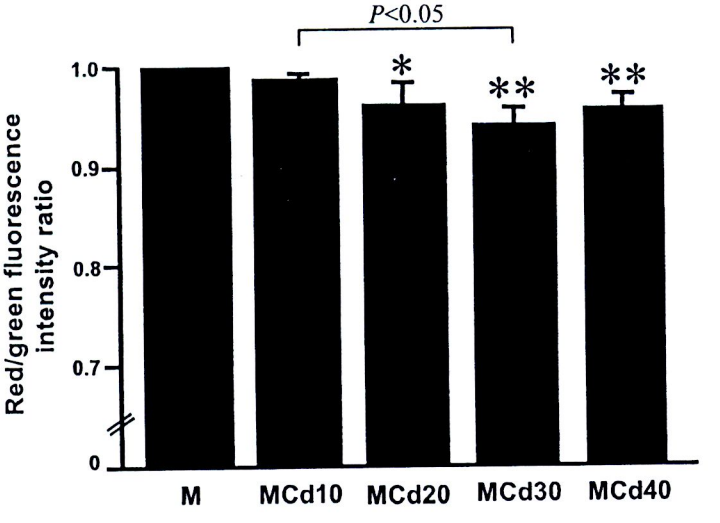

Figure 3-3 Effects of various concentrations of cadmium on mitochondrial membrane potential changes. Values are means ± SEM of 8 experiments. M, control mitochondria; MCd (10, 20, 30, 40), mitochondria treated with 10, 20, 30, and 40 μM $CdCl_2$, respectively. *P<0.05, **P<0.01 vs. M group.

Based on the above results, the concentration of $CdCl_2$ at 30 μM was chosen to be used in all the rest of the present study as it consistency produced a moderate degree of mitochondrial dysfunction.

3.2 Effects of CAPE on Mitochondrial Function under Normal Condition

As shown in Figure 3-4A and 3-4B, the absorbance of all CAPE-treated groups was similar to that of the untreated control for the duration of experiment.

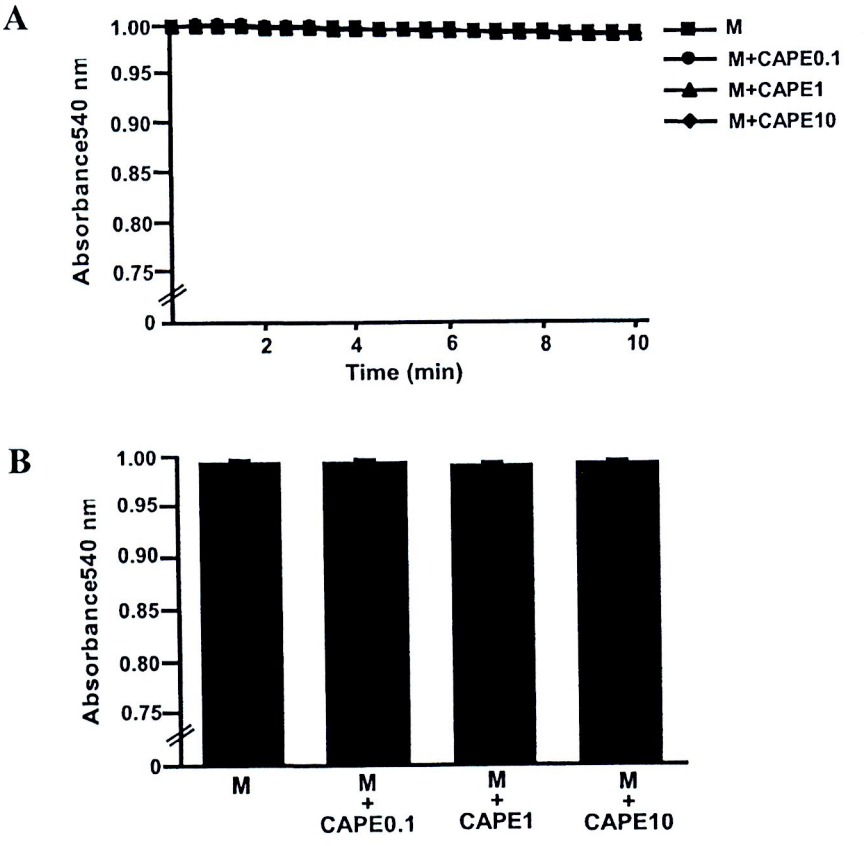

Figure 3-4 Effects of various concentrations of CAPE on mitochondrial swelling under normal condition (A) kinetics (B) endpoint. Values are means ± SEM from 8 experiments. M, control mitochondria; M+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively. *P<0.05 vs. M group.

Application of CAPE at the concentrations of 0.1 as well as 1 μM to the mitochondrial suspension resulted in a tendency to lower the basal production of ROS by mitochondria though the magnitude of reduction did not reach statistical significance (Figure 3-5). At the concentration of 10 μM, however, CAPE obviously decreased the amount of ROS as compared to the level recorded from the untreated mitochondria at basal state.

Effects of various concentrations of CAPE on mitochondrial membrane potential changes under normal condition were depicted in Figure 3-6. It appeared that CAPE had no meaningful effects on the mitochondrial membrane potential changes, irrespective of the concentrations used.

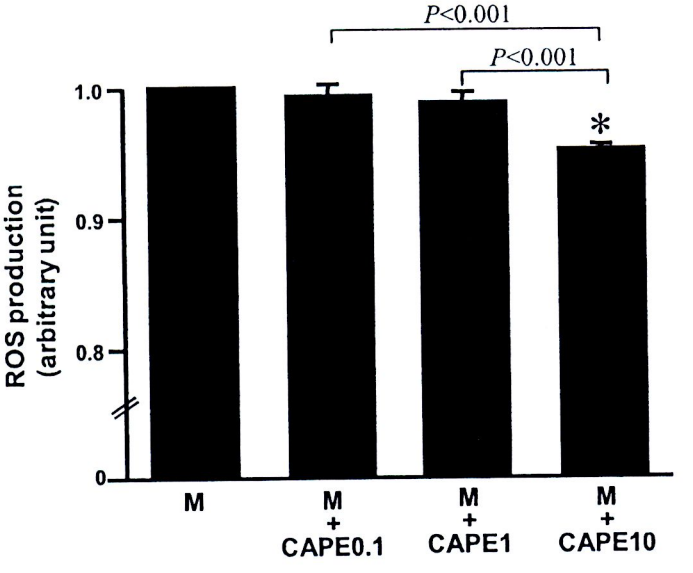

Figure 3-5 Effects of various concentrations of CAPE on mitochondrial ROS production under normal condition. Values are means ± SEM from 8 experiments. M, control mitochondria; M+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively. *P<0.001 vs. M group.

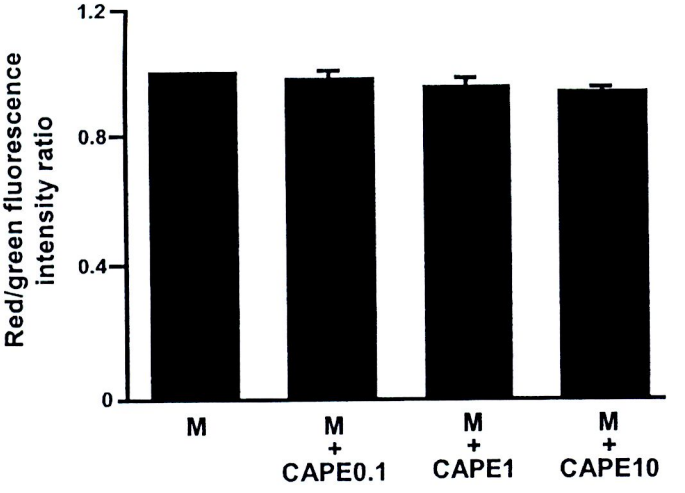

Figure 3-6 Effects of various concentrations of CAPE on mitochondrial membrane potential changes under normal condition. Values are means ± SEM of 8 experiments. M, control mitochondria; M+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively. *P<0.05 vs. M group.

3.3 Effects of CAPE on Mitochondrial Function Following Cadmium Exposure

A rapidly and significantly decreased mitochondrial absorbance at 540 nm was detected in $CdCl_2$-treated mitochondria during the measurement period of up to 10 min (Figure 3-7A and 3-7B). Addition of CAPE prior to $CdCl_2$ treatment dose-dependently attenuated cadmium-induced a reduction in mitochondrial absorbance, although to some extent. A significant increment of ROS (Figure 3-8) as well as a decline in $\Delta\Psi m$ (Figure 3-9) noted in cadmium-intoxicated mitochondria was also blunted upon co-administration of CAPE with $CdCl_2$. Interestingly, CAPE at the concentrations of 1 and 10 μM were able to restore the alterations in mitochondrial ROS and $\Delta\Psi m$ caused by cadmium to the values that were comparable with the control.

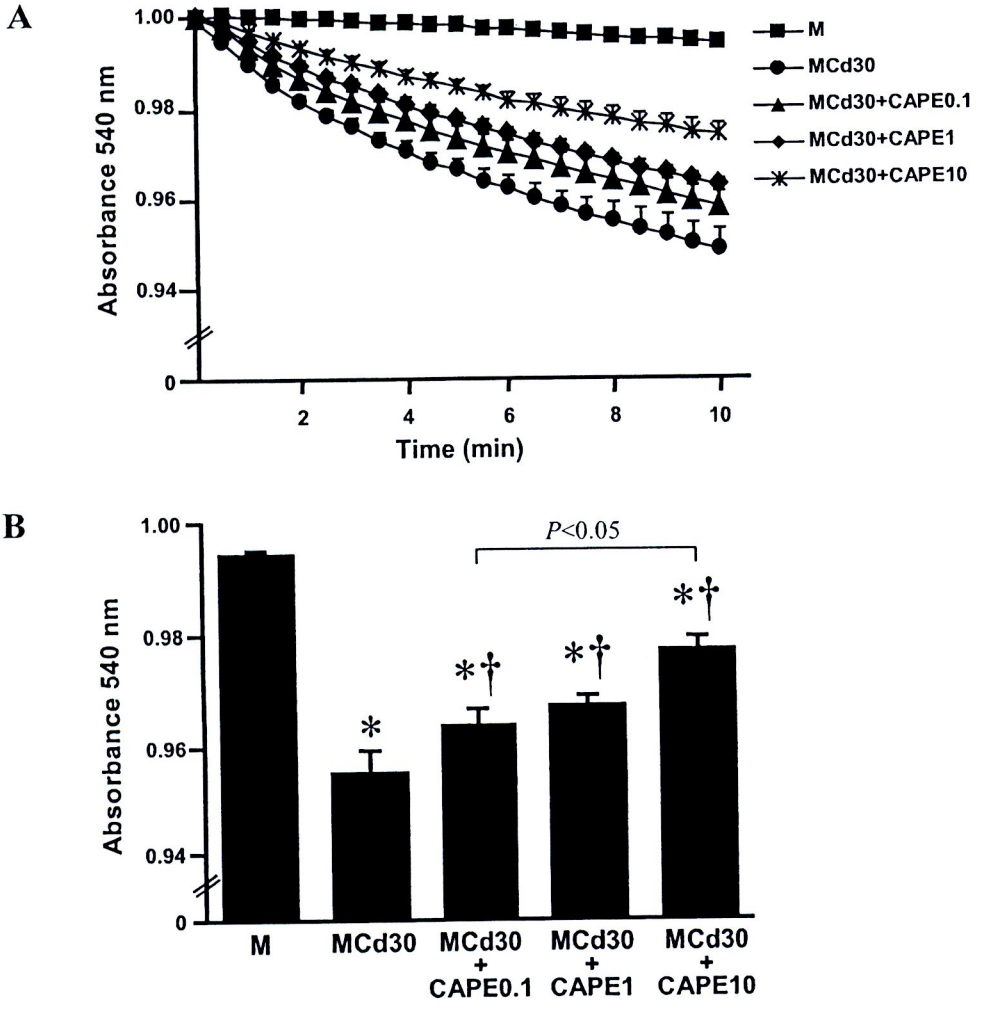

Figure 3-7 Effects of CAPE on kidney mitochondrial swelling following cadmium exposure (A) kinetics (B) endpoint. Values are means ± SEM from 8 experiments. M, control mitochondria; MCd30, mitochondria treated with 30 μM $CdCl_2$; MCd30+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively, prior to $CdCl_2$ treatment. *P<0.001 vs. M group, †P<0.001 vs. MCd30 group.

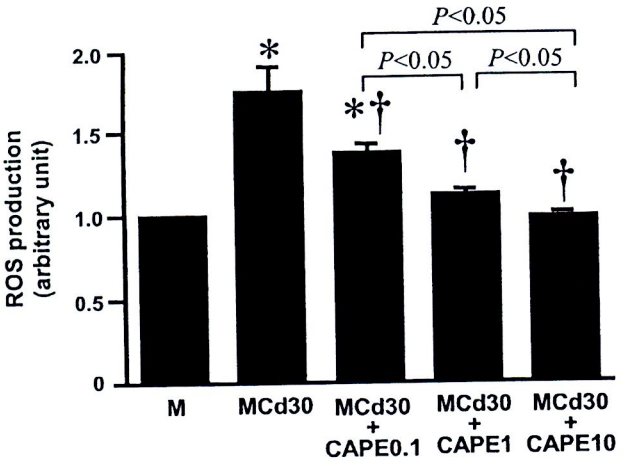

Figure 3-8 Effects of CAPE on mitochondrial ROS production following cadmium exposure. Values are means ± SEM from 8 experiments. M, control mitochondria; MCd30, mitochondria treated with 30 μM $CdCl_2$; MCd30+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively, prior to $CdCl_2$ treatment. *P<0.001 vs. M group, †P<0.001 vs. MCd30 group.

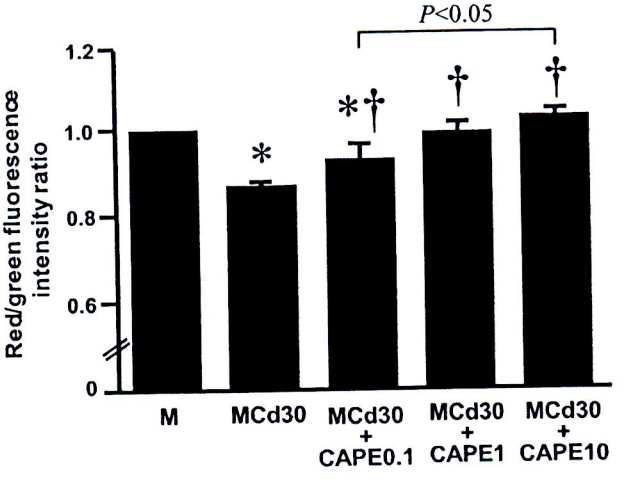

Figure 3-9 Effects of CAPE on mitochondrial membrane potential changes following cadmium exposure. Values are means ± SEM from 8 experiments. M, control mitochondria; MCd30, mitochondria treated with 30 μM $CdCl_2$; MCd30+CAPE (0.1, 1, 10), mitochondria treated with 0.1, 1, and 10 μM CAPE, respectively, prior to $CdCl_2$ treatment. *P<0.001 vs. M group, †P<0.001 vs. MCd30 group.

3.4 Effects of Cadmium and CAPE on Mitochondrial Oxidative Stress

Mitochondria exposed to $CdCl_2$ showed an obvious increase in MDA (Figure 3-10A) associated with a marked reduction in GSH (Figure 3-10B) levels in comparison with the non-exposed control. Pretreated mitochondria with CAPE protected against the changes in MDA and GSH resulted from $CdCl_2$ exposure. Neither the level of MDA nor GSH was significantly altered in the group of mitochondria treated with CAPE alone compared to those in the control.

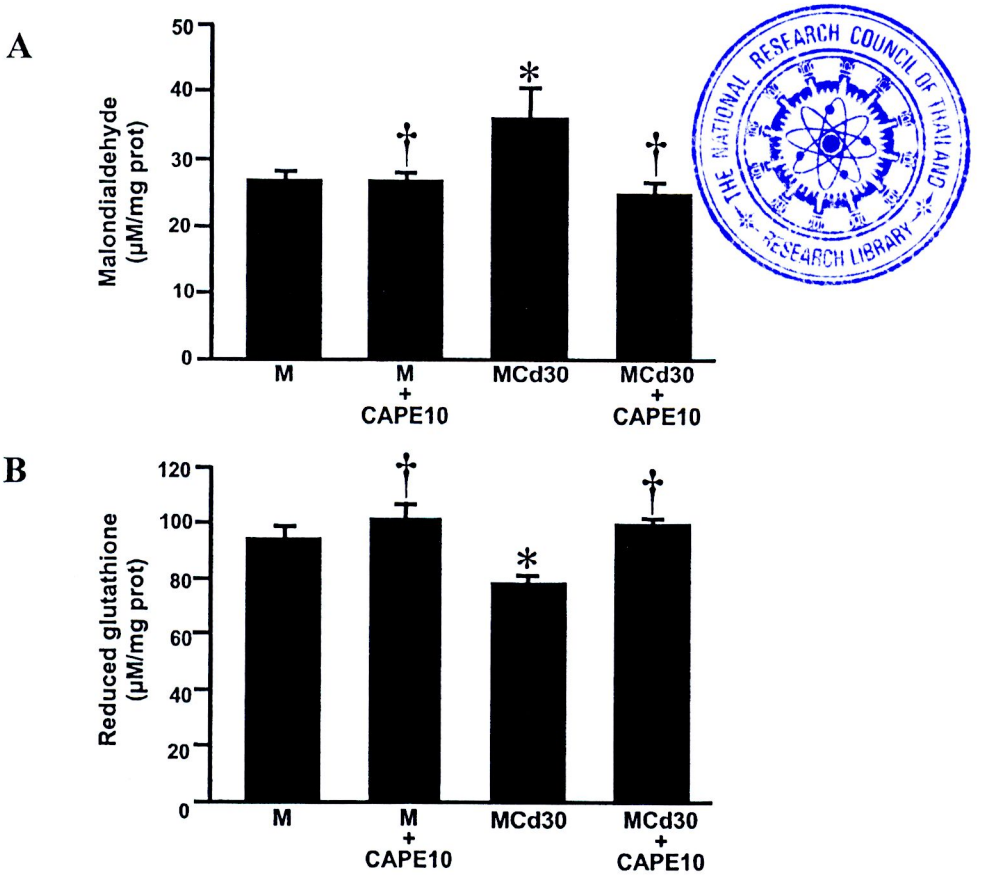

Figure 3-10 Effects of cadmium and CAPE on malondialdehyde (A) and reduced glutathione (B) levels. Values are means ± SEM from 8 experiments. M, control mitochondria; M+CAPE10, mitochondria treated with 10 μM CAPE; MCd30, mitochondria treated with 30 μM $CdCl_2$; MCd30+CAPE10, mitochondria treated with CAPE prior to $CdCl_2$ treatment. *P<0.001 vs. M group, †P<0.001 vs. MCd30 group.

3.5 Effects of Cadmium and CAPE on Mitochondrial Structure

Electron microscopic examination revealed that the untreated mitochondria maintained their integrity, with classical ultrastructure containing densely packed cristae and matrix (Figure 3-11A). CAPE itself did not modify the mitochondrial configuration (Figure 3-11B). Following $CdCl_2$ exposure, mitochondria appeared swollen, less dense matrix, and, in some mitochondria, ruptured of the membrane fragmented or disappeared cristae (Figure 3-11C). These structural alterations induced by cadmium were ameliorated in mitochondria pretreated with CAPE (Figure 3-11D).

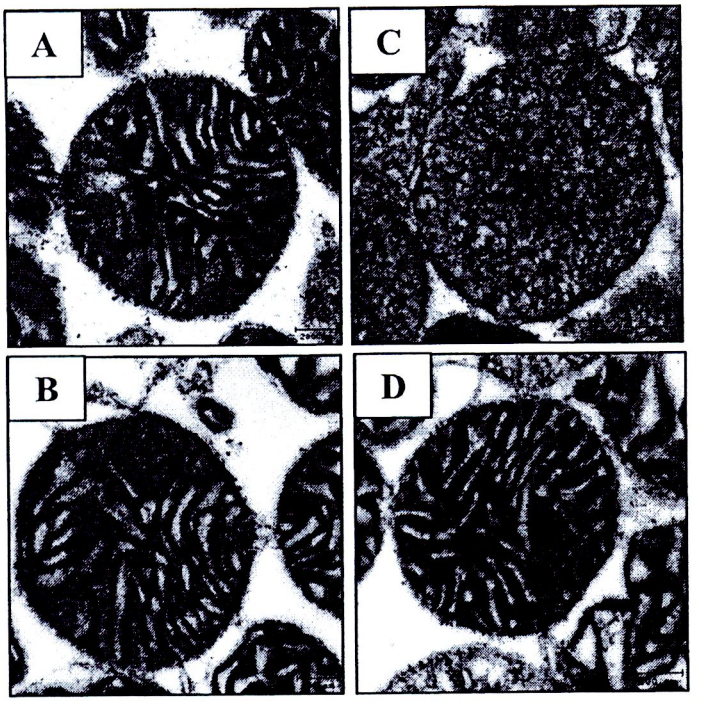

Figure 3-11 Electron micrograph showing the effects of cadmium and CAPE on mitochondrial ultrastructural changes. A, control mitochondria; B, mitochondria treated with 10 μM CAPE; C, mitochondria treated with 30 μM $CdCl_2$; D, mitochondria treated with CAPE prior to $CdCl_2$. Original magnification: 15000x.