In Vitro Cytotoxicity of 4-Methylcatechol in Murine Tumor Cells: Induction of Apoptotic Cell Death by Extracellular Pro-Oxidant Action

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JPET Fast Forward. Published on April 3, 2003 as DOI: 10.1124/jpet.103.050351
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a) Running title: Pro-oxidant action of 4-methylcatechol on tumor cells

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c) Text pages = 27

Figures = 7

References = 34

Words in the Abstract = 240

Words in the Introduction = 650

Words in the Discussion = 930

d) Abbreviations:

DMEM, Dulbecco's modified Eagle's medium

Ac-DEVD-AMC, Acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin

GSH, reduced-form glutathione

e) Recommended section: Toxicology

JPET Fast Forward. Published on April 3, 2003 as DOI: 10.1124/jpet.103.050351
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ABSTRACT

Assessment of the *in vitro* cytotoxicity has recently been become popular as a primary screening method for evaluating the antitumor activities of various chemicals and natural substances. For example, quercetin and related phenolic compounds, presented in teas, wines, and other plant products, have been shown to cause their cytotoxic effects on tumor cells in culture, proposing their protective effects against the development of cancer. However, 4-methylcatechol, a metabolite produced in the intestinal tract after ingestion, has been shown to cause the promotion rather than the suppression of tumor in rat stomach despite its *in vitro* cytotoxic activity. To address the inconsistency between its *in vivo* and *in vitro* actions, the effect of 4-methylcatechol on the viabilities of murine tumor cells was examined, and 4-methylcatechol was shown to reduce their viabilities through the induction of apoptosis. In addition, since catechol compounds have been shown to have a complex mixture of pro-oxidant and antioxidant actions in the *in vitro* assay systems, the cytotoxic activity of 4-methylcatechol was reassessed in the presence of either catalase or reduced-form glutathione (GSH), and both of them were shown to protect the cells against the damage induced by 4-methylcatechol. Moreover, the generation of hydrogen peroxide was observed by incubating the drug in the growth medium with or without the cells. These findings indicate that, similar to other catechol compounds, 4-methylcatechol may induce the apoptotic death of murine tumor cells through its extracellular pro-oxidant action on the cells.

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Quercetin, catechins and related phenolic compounds are known to be widely distributed in the plant kingdom and contained in various foods and beverages, such as fruits, vegetables, wines and teas. Recently, these phenolic compounds have been shown to exert the potential beneficial effects on human health, such as protective effects against the development of cancer and cardiovascular diseases, through their antioxidative, tissue-protective and carcinostatic actions (Ames et al., 1993; Bors et al., 1990; Diplock et al., 1998; Wang et al., 1998). In addition to these actions, polyphenolic flavonoid compounds have been shown to have the capacity to inhibit hepatocellular cholesterol biosynthesis (Gebhardt, 1998). However, the most of studies on their actions have been carried out using the appropriate *in vitro* experimental systems, which are considered to be favorable for investigating the underlying molecular mechanisms, and hence it seems necessary to precisely characterize and compare the actions of these phenolic compounds in the *in vivo* and the *in vitro* systems for evaluating their beneficial health effects.

Since polyphenolic flavonoid compounds have been shown to be rapidly metabolized in the intestinal tract after ingestion (Gee et al., 2000; Graefe et al., 1999; Hollman and Katan, 1999; Wiseman, 1999), it seems quite possible that the beneficial effects of these compounds may be attributed fully or partly to the biological actions of their metabolites in experimental animals and humans. Therefore, the biological activities of their possible metabolites have been investigated in comparison with those of the parent compounds. For example, the antioxidant activities and the inhibitory actions on cholesterol biosynthesis of quercetin and its possible metabolites have been studied, and 4-methylcatechol, also called 3,4-dihydroxytoluene and well known as a metabolite of quercetin produced in the intestinal tract after ingestion, has been shown to inhibit both lipid peroxidation and cholesterol biosynthesis as potent as the parent phenolic compounds in the

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primary cultures of rat hepatocytes (Gläßer et al., 2002). On the other hand, the differences in the biological actions between the parent flavonoid compounds and their metabolites have been reported as well, and the cytotoxic effects of these compounds are well known as such cases. Flavonoid compounds have been shown to cause their cytotoxic effects on malignant cells in culture (Chen et al., 1998; Paschka et al., 1998; Richter et al., 1999; Russo et al., 1999; Saeki et al., 1999; Sergediene et al., 1999), thus proposing their protective effects against the development of cancer. In addition, 4-methylcatechol, a metabolite of flavonoid compounds and also known to be produced by the biodegradation of toluene in liver, has been shown to cause the cytotoxic effect on several types of the cells in culture (Ito et al., 1981; Shen, 1998; Shen et al., 2000). However, despite the *in vitro* cytotoxicity, this compound has been reported to show the carcinogenic rather than the carcinostatic activity in rat stomach (Asakawa et al., 1994; Furihata et al., 1993; Hirose et al., 1988; Hirose et al., 1989). In our preliminary studies, the effect of 4-methylcatechol on the rate of tumor growth in mice inoculated with B16-F10 melanoma cells was examined to assess its carcinostatic action, but this compound failed to reveal any substantial antitumor activity in these tumor-bearing animals. Thus, the *in vitro* cytotoxicity of phenolic compounds is considered to be not always a positive index of the *in vivo* carcinostatic action.

Recently, the cytotoxic effects of various chemicals and natural substances on malignant tumor cells in culture have been extensively studied as a primary screening for their antitumor activities, and hence it seems important and necessary to previously confirm the connection between the *in vitro* cytotoxic and the *in vivo* antitumor activities. In this respect, it seems helpful for assessment of the antitumor activities of phenolic compounds to understand the reason for the inconsistency between the *in vivo* and *in vitro* effects of 4-methylcatechol. For this purpose, the *in vitro* cytotoxic

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effect of 4-methylcatechol on murine tumor cells was further investigated.

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Materials and Methods

Materials. Mouse B16-F10 melanoma cells (CRL-6475), mouse LL/2 Lewis lung carcinoma cells (CRL-1642), rat C6 glioma cells (CCL-107) and rat PC12 pheochromocytoma cells (CRL-1721) were obtained from the American Type Culture Collection (Rockville, MD, USA). 4-methylcatechol was purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Neutral red solution, catalase, reduced-form glutathione (GSH), acridine orange, propidium iodide and Acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin (Ac-DEVD-AMC) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). PeroXOquant^{TM} Quantitative Peroxide Assay Kit (aqueous-compatible formulation) was from Pierce Biotechnology (Rockford, IL, USA). Other chemicals used were commercially available reagent grade or ultrapure grade.

Cell Culture. Cells were maintained as monolayer cultures on a 60-mm culture dish in the growth medium [Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated bovine calf serum, 5% equine serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin and 50 μg/ml of gentamycin sulfate] at 37^{o}C in a humidified incubator containing a 95% air - 5% CO_2 atmosphere.

Cell Growth and Viability. For the growth determination, the cells were plated on a 24-well cluster plate at a density of 2 x 10^4 cells/well, and cultured for 24 h to allow the cells to attach to the bottom of the plastic plate. The medium was replace with the fresh growth medium, and then cultured with or without 4-methylcatechol for different periods. For the determination of cell

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viability, the cells were plated at a density of 5 x 10^4 cells/well, and cultured for 48 h. The medium was replaced with the serum-free medium [DMEM containing 50 units/ml of penicillin, 50 μg/ml of streptomycin and 50 μg/ml of gentamycin sulfate, 5 μg/ml of insulin, 5 μg/ml of transferrin and 5 ng/ml of sodium selenite], and the cells were cultured for 24 h to arrest the cell growth, and then treated with various concentrations of 4-methylcatechol for 24 h in the serum-free medium.

Cell growth and viability were determined by measuring the amount of neutral red taken up into the cells as reported previously (Fautz et al., 1991; Morita et al., 1999; Morita and Wong, 2000). Briefly, the cells were washed with saline solution, and incubated in 0.5 ml of DMEM containing neutral red (50 μg/ml) for 2 h in a humidified incubator. Then, the cells were washed with saline solution, and extracted with acidified ethanol solution (50% ethanol - 1% acetic acid) for 20 min at room temperature with constant gentle shaking. The amount of neutral red taken up into the cells was spectrophotometrically determined by measuring the optical density at 540 nm, and the cell viability was calculated as a percentage of the control.

Cell viability was also determined by measuring the amount of total protein. The cells were cultured for 24 h as described above, and washed with saline, and then dissolved in 0.5 ml of 1 M NaOH for 20 min at room temperature with gentle shaking. The amount of total protein in an aliquot of the extract was determined by the method of Bradford (Bradford, 1976) using bovine immunoglobulin G as a standard.

Numbers of viable and dead cells were determined using a trypan blue dye exclusion method. Briefly, the cells were cultured on a 35-m/m dish, and exposed to 4-methylcatechol in the growth medium. Both attached and floating cells were collected by trypsinization, and an aliquot of the cells were mixed with an equal volume of trypan blue solution. The cells excluding dye (viable

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cells) and those taking up dye (dead cells) were counted in duplicate using a hemocytometer, and the numbers of these cells were expressed as the percent of total cell number.

Apoptotic Cell Damage. For the fluorescence cytochemical study, the cells were plated on a poly-D-lysine-coated 35-m/m culture dish at a density of 1 x 10^4 cells/dish, and treated with 4-methylcatechol for 24 h as described above. The medium was removed by aspiration, and the cells were rinsed with PBS, and fixed with methanol-acetic acid solution (3 : 1) for 1 h at room temperature. The fixed cells were incubated in Fluorescence-Dye solution [5 μg/ml of propidium iodide and 50 μg/ml of acridine orange in phosphate-buffered saline] for 1 h at room temperature, and then examined by an inverted fluorescence microscope.

For the DNA fragmentation analysis, the cells were plated on a 60-mm culture dish at a density of 5 x 10^5 cells/dish, and treated with 4-methylcatechol for 24 h as described above. The cells attached at the bottom were scraped off, and collected together with unattached cells by centrifuging at 1,500 x g for 5 min at 4°C. The DNA was prepared from the pelleted cells, and applied to a 1.8% agarose gel containing 0.5 μg/ml of ethidium bromide for the electrophoretic DNA analysis as described previously (Morita et al., 1999; Morita and Wong, 2000).

For the caspase-3 assay, the cells were plated on a 24-well cluster plate at a density of 5 x 10^4 cells/well, and treated with 4-methylcatechol for 6 h as described above. The medium was removed, and the cells were rinsed with ice-cold saline, and then lysed with the extraction buffer [50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40] followed by a freeze-thaw cycle. The lysate was passed 20 - 25 times through a blue pipetman tip, and then centrifuged at 150,000 rpm (20,000 x g) for 20 min at 4°C. The caspase-3 activity in the supernatant fraction was

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then determined as reported previously (LaRue et al., 2000). An aliquot (50 µl) of the supernatant fraction was mixed with 150 µl of the reaction buffer [20 mM HEPES, 10% glycerol, 2 mM DTT] containing 10 µl of 1 mM Ac-DEVD-AMC in an ELISA plate micro-well. The mixture was incubated for 2 h at room temperature under the light-protecting conditions, and the fluorescence intensity was measured at 365 nm (Ex) - 450 nm (Em) using a microplate reader, and the enzyme activity was expressed as a percentage of the control.

Hydrogen Peroxide Generation. Cells were plated on one half of a 24-well cluster plate at a density of 5 x 10^4 cells/well, and the growth medium only was added to the other half. The cells were cultured as described above, and incubated with various concentrations of 4-methylcatechol for 2 h. The medium was collected, and centrifuged at 15,000 rpm (20,000 x g) for 20 min to remove the floating cells and cell fragments. The concentration of hydrogen peroxide in the supernatant fraction was determined based on the method reported previously (Nourooz-Zadeh et al., 1994) using PeroXOquant^{TM} Quantitative Peroxide Assay Kit.

Data Analysis. Results were presented as the mean $\pm$ S.E. Data were analyzed by an analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A p value of <0.05 was accepted as a statistically significant difference.

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Results

To examine the effect of 4-methylcatechol on the growth of murine tumor cells, mouse B16 melanoma cells were treated with 100 μM of the drug for different time period, and the number of viable cells was then determined by measuring the amount of neutral red taken up into the cells. As shown in Fig. 1, the uptake of neutral red into the cells was markedly reduced by treatment of the cells with 4-methylcatechol, and an approximately 50% reduction of the uptake was observed at 24 and 48 h after the exposure to the drug. In addition, the significant reduction of neutral red uptake was still observed at 72 h, but its extent (approximately 20% reduction) was relatively small in comparison with that observed at 24 or 48 h. Thus, 4-methylcatechol was clearly shown to reduce the uptake of neutral red into B16 cells, but it seemed still necessary to elucidate whether the reduction of growth rate observed here might directly reflect the suppression of cell proliferation or the induction of cell death.

To characterize the effect of 4-methylcatechol on mouse B16 melanoma cells, the cells were exposed to 100 μM of the drug for 24 h, and the numbers of viable and dead cells were determined. As shown in Fig. 2, the percentage of viable cells was slightly reduced by treatment of these cells with 4-methylcatechol (approximately 91 and 76% in the control and treated groups, respectively), whereas the percentage of dead cells in the treated group was approximately 260% higher than that in the control. Consistent with the results presented in Fig. 1, an approximately 50% reduction of neutral red uptake was also observed in the separate experiments (data not shown). Even though the discrepancy in the reduction of cell viabilities estimated by the trypan blue exclusion and neutral red uptake methods was observed, these results seemed to indicate that 4-methylcatechol might be

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able to induce the cytotoxic damage to B16 cells in culture.

To further characterize the cytotoxic effect of 4-methylcatechol on mouse B16 melanoma cells, the cell growth was arrested by maintaining them in the serum-deprived culture medium for 24 h, and the effect of 4-methylcatechol on these cells was examined under the serum-free conditions. As shown in Fig. 3, the reduction of neutral red uptake was observed in a manner dependent on the concentration of 4-methylcatechol. In addition, 4-methylcatechol also caused the reduction of total protein amount, similar in extent to the reduction of dye uptake. These results indicated that the reduction of neutral red uptake was not due to the impairment of dye transport across the plasma membrane of these cells, but directly reflected a decrease in the number of viable cells, providing evidence that 4-methylcatechol could exert the cytocidal rather than the cytostatic action on B16 cells in culture.

To test the susceptibilities of other murine tumor cells to the cytotoxic effect of 4-methylcatechol, mouse LL/2 Lewis lung carcinoma cells, rat C6 glioma cells and rat PC12 pheochromocytoma cells were exposed to different concentrations of the drug for 24 h under the serum-free culture conditions, and their viabilities were determined by measuring the uptake of neutral red into these cells. As shown in Fig. 4, these cells were susceptible to 4-methylcatechol with the slight differences in their susceptibities. The cytotoxic effect of 4-methylcatechol on C6 cells and LL/2 cells was almost similar to that on B16 cells, and PC12 cells showed the slightly less but substantially similar susceptibility to the drug as compared with that of B16 cells. These results were thought to indicate that 4-methylcatechol might induce the damage to murine tumor cells, resulting in the cell death through the common process to these cells.

To investigate the manner of cell death induced by 4-methylcatechol, B16 melanoma cells were

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exposed to the drug, and the assessment of apoptotic cell death was then carried out using a fluorescence dye staining, a DNA fragmentation analysis and a caspase-3 assay. Morphological study showed that B16 cells were uniformly stained with acridine orange (green color) in the control group, and the cells with dots of condensed chromatin were observed in the treated group, whereas the cells stained with propidium iodide (red color) were not detected in both the control and treated groups (Fig. 5a). Electrophoretic analysis also showed that 4-methylcatechol induced the degradation of DNA into nucleosomic fragments at the concentration range required to reduce the cell viability (Fig. 5b). In the control, there was no fluorescent signal observed in the region of agarose-gel ranging from 100 bp to 500 bp, while the signal appeared in this region by exposure of the cells to 4-MC. This signal was intensified by increasing the drug concentration from 50 to 100 μM, but the signal at higer dose (250 μM) was less intense in comparison with that observed at lower dose (100 μM). In contrast, the fluorescence in the region ranging lower than 100 bp increased according to the drug concentration. Therefore, it seemed possible that the degradation of DNA to nucleosomic fragments might be induced by the exposure to 4-MC, and the further degradation of DNA fragments could occur in the presence of higher concentration of the drug. Moreover, the elevation of caspase-3 activity was observed by short-term exposure of the cells to the drug (Fig. 5c). These results indicated that 4-methylcatechol reduced the viability of B16 cells as a result of inducing the apoptotic cell death under the conditions used here.

Phenolic compounds are generally known to show not only their antioxidative effects but also pro-oxidant actions under the *in vitro* assay conditions, and hence it seemed possible that 4-methylcatechol might exert its pro-oxidant action on the cells in culture. To test this possibility, the cytotoxicity of 4-methylcatechol on B16 melanoma cells was assessed in the presence of a

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hydroperoxide scavenger, catalase or GSH. As shown in Fig. 6, the reduction of cell viability induced by 4-methylcatechol was blocked by catalase and GSH at the concentrations of 500 units/ml and 2 mM, respectively. In addition, the generation of hydrogen peroxide during the incubation of 4-methylcatechol in the culture of B16 cells was determined to directly examine its pro-oxidant action. As shown in Fig. 7, the concentration of hydrogen peroxide in the cell culture was increasing in a concentration-dependent manner, and the substantial generation of hydrogen peroxide was also observed even by incubating the drug in the medium without the cells. These results indicated that 4-methylcatechol could be oxidized in the cell culture and exerted pro-oxidant action on the cells.

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Discussion

Cytotoxic effect of 4-methylcatechol on murine tumor cells in culture was examined by measuring their growth rate using the neutral red uptake method, and the growth of mouse B16 melanoma cells was first shown to be suppressed by the presence of this drug in the culture medium (Fig. 1). However, despite a considerable increase in the number of dead cells, the trypan blue exclusion method used here failed to show a significant decrease in the number of viable cells after exposing the cells to 4-methylcatechol (Fig. 2). Since damaged cells, particularly cells in the early stages of apoptosis, are generally known to retain their abilities to exclude the vital dye and may often score as viable cells, it seems highly probable that the percentages of viable and dead cells obtained here may not correctly reflect the actual numbers of these cells. Then, the effect of 4-methylcatechol on the viability of B16 melanoma cells was examined by measuring the amount of total protein as well as the uptake of neutral red into the cells under the conditions in which the cell growth was arrested to exclude a possible influence on the cell proliferation. Consequently, 4-methylcatechol was shown to markedly reduce the cell viability in a concentration-dependent manner (Fig. 3), and hence the suppression of cell growth induced by this drug was shown to be due to the induction of cell death rather than the inhibition of cell proliferation, thus providing evidence for the *in vitro* cytotoxicity of 4-methylcatechol in malignant tumor cells. Furthermore, the effect of 4-methylcatechol on other murine tumor cells, such as mouse LL/2 Lewis lung carcinoma cells, rat C6 glioma cells and rat PC12 pheochromocytoma cells, was examined under the same conditions, and the reductions of their viabilities induced by 4-methylcatechol were shown to be almost similar in extent to that observed in B16 melanoma cells (Fig. 4). Thus, it seems

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obvious that 4-methylcatechol can exert the cytotoxic effect on murine tumor cells, regardless of their original tissues and species, and probably their malignancies as well.

The viabilities of murine tumor cells were shown to be reduced by the exposure of these cells to 4-methylcatechol in a concentration-dependent manner, and the cytotoxic effect was observed to be non-selective about their original tissues and species. Further studies showed that characteristics of the apoptotic cell damage, such as chromatin condensation, DNA fragmentation and caspase-3 activation, were observed in B16 melanoma cells treated with 4-methylcatechol (Fig. 5). Together, these observations clearly indicate that 4-methylcatechol induces the apoptotic damage to murine tumor cells in culture, resulting in the reduction of their viabilities under the *in vitro* experimental conditions. In general, the apoptotic cell death is considered to be mostly induced by oxidative insults, and hence it seems conceivable that the cytotoxic effect of 4-methylcatechol observed here may be the result of the oxidative damage to the cells. Then, the effect of 4-methylcatechol on the viability of B16 melanoma cells was again examined in the presence of catalase and GSH, and these hydroperoxide scavengers were shown to successfully protect the cells against the cytotoxic effect of this drug (Fig. 6). Therefore, it seems likely that the cytotoxicity of 4-methylcatechol observed here may be due to the toxic effect of hydrogen peroxide on B16 melanoma cells in culture.

Previously, quercetin and related phenolic compounds containing a catechol moiety in their chemical structures have been shown to be oxidized, resulting in lipid peroxidation under the *in vitro* certain assay conditions (Galati et al., 1999; Laughton et al., 1989; Yamanaka et al., 1997). Recent studies have also shown that these compounds can be oxidized and can rapidly generate hydrogen peroxide in commonly used cell culture media (Long et al., 2000). Moreover, both DOPA

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and dopamine have been shown to undergo oxidation to generate hydrogen peroxide and semiquinones/quinones by interacting with commonly used culture media (Clement et al., 2002). Based on these previous findings, it seems reasonable to consider that catechol compounds can exert the oxidative damage to the cells as a result of generating hydrogen peroxide in the culture medium, and hence the generation of hydrogen peroxide from 4-methylcatechol was examined by incubating the drug in the medium with or without B16 melanoma cells. Considerable amount of hydrogen peroxide was shown to be generated by incubating 4-methylcatechol in the cell culture, and the substantial generation of hydrogen peroxide was also observed even by incubating the drug in the culture medium without the cells (Fig. 7). In addition to the generation of hydrogen peroxide observed here, it seems conceivable that, similar to DOPA and dopamine, 4-methylcatechol may have the property of generating semiquinones/quinones by interacting with cells and/or unidentified constituent(s) in the culture medium. Thus, it is likely that 4-methylcatechol can be oxidized and generate hydrogen peroxide, and probably semiquinones/quinones, in the cell culture, resulting in the oxidative damage to murine tumor cells *in vitro*.

In summary, 4-methylcatechol is shown to induce the apoptotic damage to murine tumor cells as a result of generating hydroperoxides in the cell culture, suggesting that the cytotoxic effect of this drug may be attributed to its pro-oxidant action on the cells. This may be able to account for the discrepancy between the *in vitro* cytotoxic and the *in vivo* antitumor activities of 4-methylcatechol and its derivatives. Recently, as a primary screening for the antitumor activity, the cytotoxic effects of various compounds on malignant tumor cells have been studied using the *in vitro* culture system. However, the results presented here suggest that caution is required when the *in vitro* cytotoxicity is assessed to predict the carcinostatic actions of phenolic compounds *in vivo*.

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Figure legends

Fig. 1. Effect of 4-methylcatechol on growth of mouse B16 melanoma cells in culture. Cells were cultured in the growth medium with or without 100 μM of 4-methylcatechol for different time periods, and a number of the viable cells was then determined by measuring neutral red uptake into the cells as described in the text. Values are the mean ± S.E. (n = 8). *significantly different from the control ($p<0.05$).

Fig. 2. Changes in viable and dead cell numbers by treatment of mouse B16 melanoma cells with 4-methylcatechol. Cells were exposed to 100 μM of 4-methylcatechol for 24 h, and the percentages of viable and dead cells were then determined using the trypan blue exclusion method as described in the text. Results were expressed as the percent of total cell number. Values are the mean ± S.E. (n = 6). *significantly different from the control ($p<0.05$).

Fig. 3. Effect of 4-methylcatechol on viability of mouse B16 melanoma cells in culture. Cells were exposed to the different concentrations of 4-methylcatechol for 24 h, and the cell viability was determined by measuring both neutral red uptake into the cells and the amount of total protein in the cell lysate. Values are the mean ± S.E. (n = 8). *significantly different from the control ($p<0.05$).

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Fig. 4. Effect of 4-methylcatechol on viabilities of murine tumor cells in culture. Various murine tumor cells were exposed to the different concentrations of 4-methylcatechol for 24 h, and the cell viabilities were then determined by measuring neutral red uptake into the cells. Values are the mean ± S.E. (n = 8).

Fig. 5. Apoptotic changes induced by 4-methylcatechol in mouse B16 melanoma cells. [a] Morphological assessment: Cells were exposed to 100 μM of 4-methylcatechol for 24 h, and the fluorescence dual-staining was carried out. [b] DNA fragmentation: Cells were exposed to the different concentrations of 4-methylcatechol for 24 h, and DNA (20 μg) prepared from these cells was subjected to the electrophoretic analysis. [c] Caspase-3 assay: Cells were exposed to the different concentrations of 4-methylcatechol for 6 h, and the enzyme activity in the cell lysate was determined. Values are the mean ± S.E. (n = 8). *significantly different from the control ($p<0.05$).

Fig. 6. Protective effects of hydroperoxide scavengers on cytotoxic effect of 4-methylcatechol on mouse B16 melanoma cells in culture. Cells were exposed to 100 μM of 4-methylcatechol for 24 h in the presence of different concentrations of catalase [A] or GSH [B], and the cell viability was determined by measuring neutral red uptake into the cells. Values are the mean ± S.E. (n = 6). *significantly different from the control ($p<0.05$).

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Fig. 7. Generation of hydrogen peroxide from 4-methylcatechol in cultures of mouse B16 melanoma cells. Different concentrations of 4-methylcatechol were incubated in the cell culture or the medium alone for 2 h, and the concentration of hydrogen peroxide in the medium was measured as described in the text. Values are the mean $\pm$ S.E. (n = 6).

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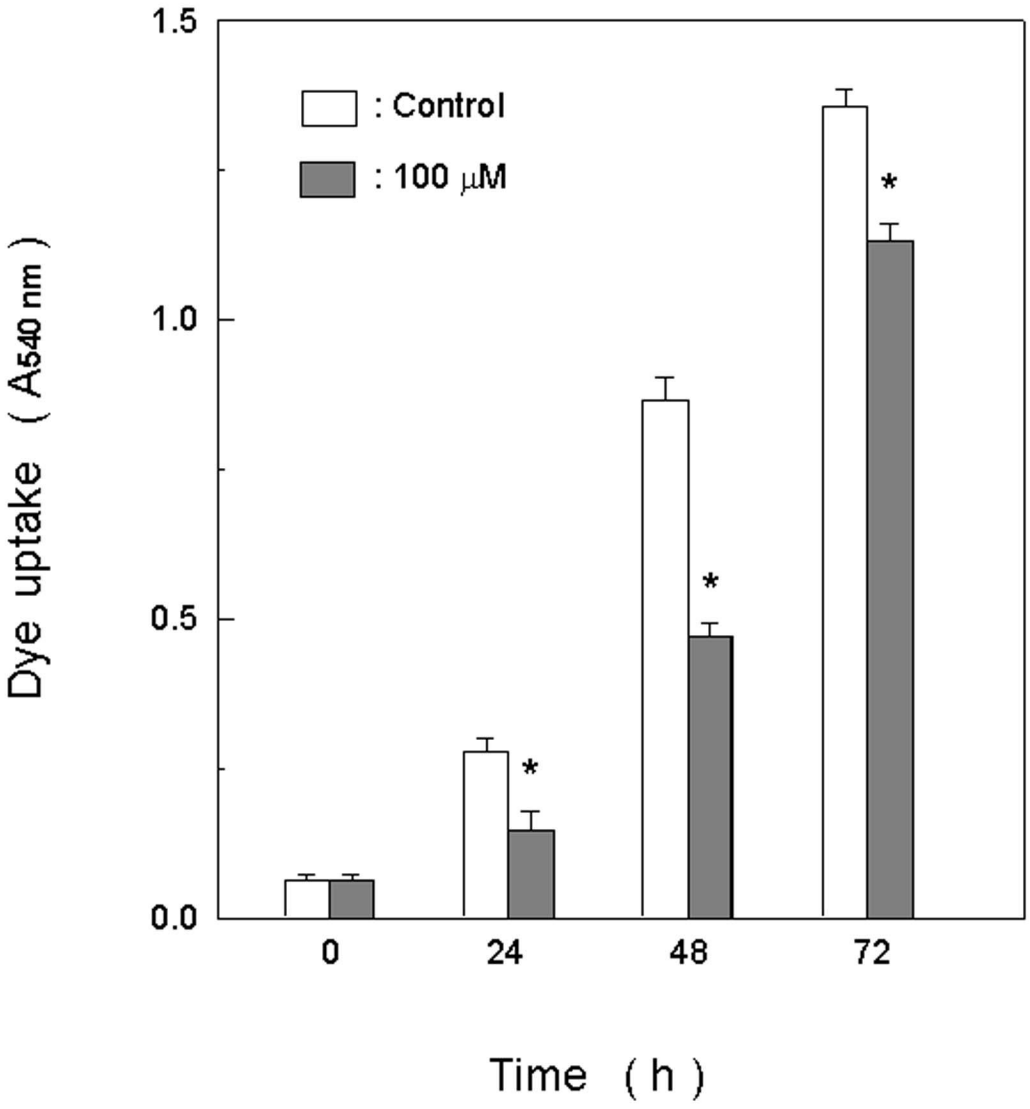

Fig. 1

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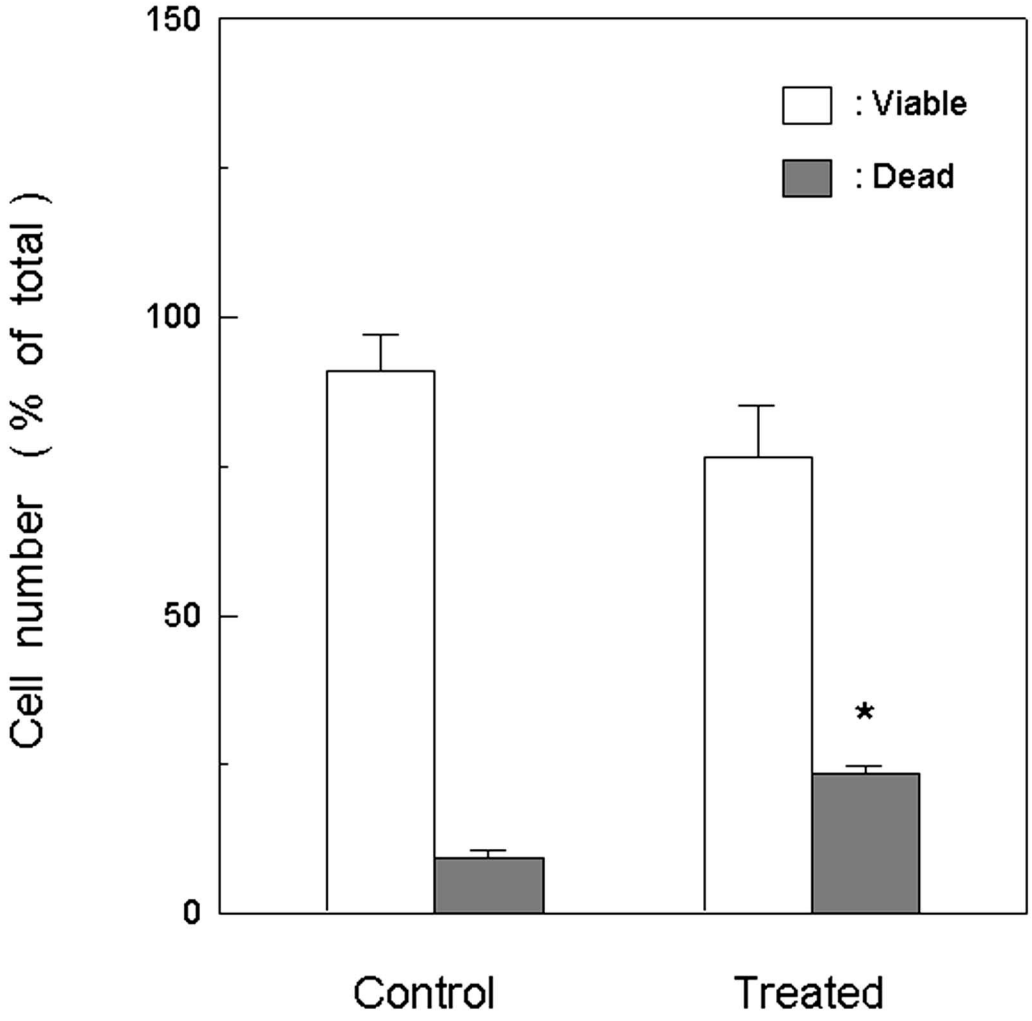

Fig. 2

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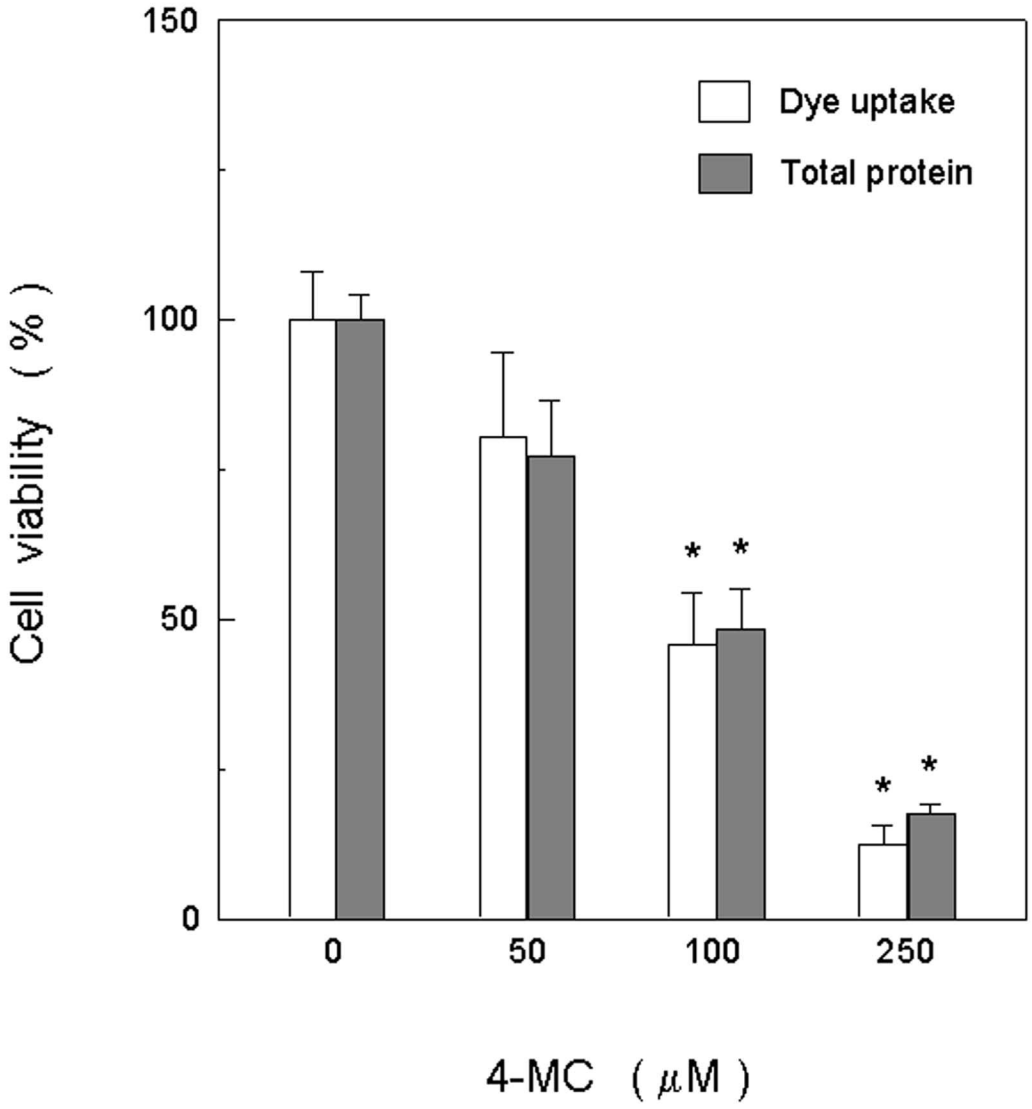

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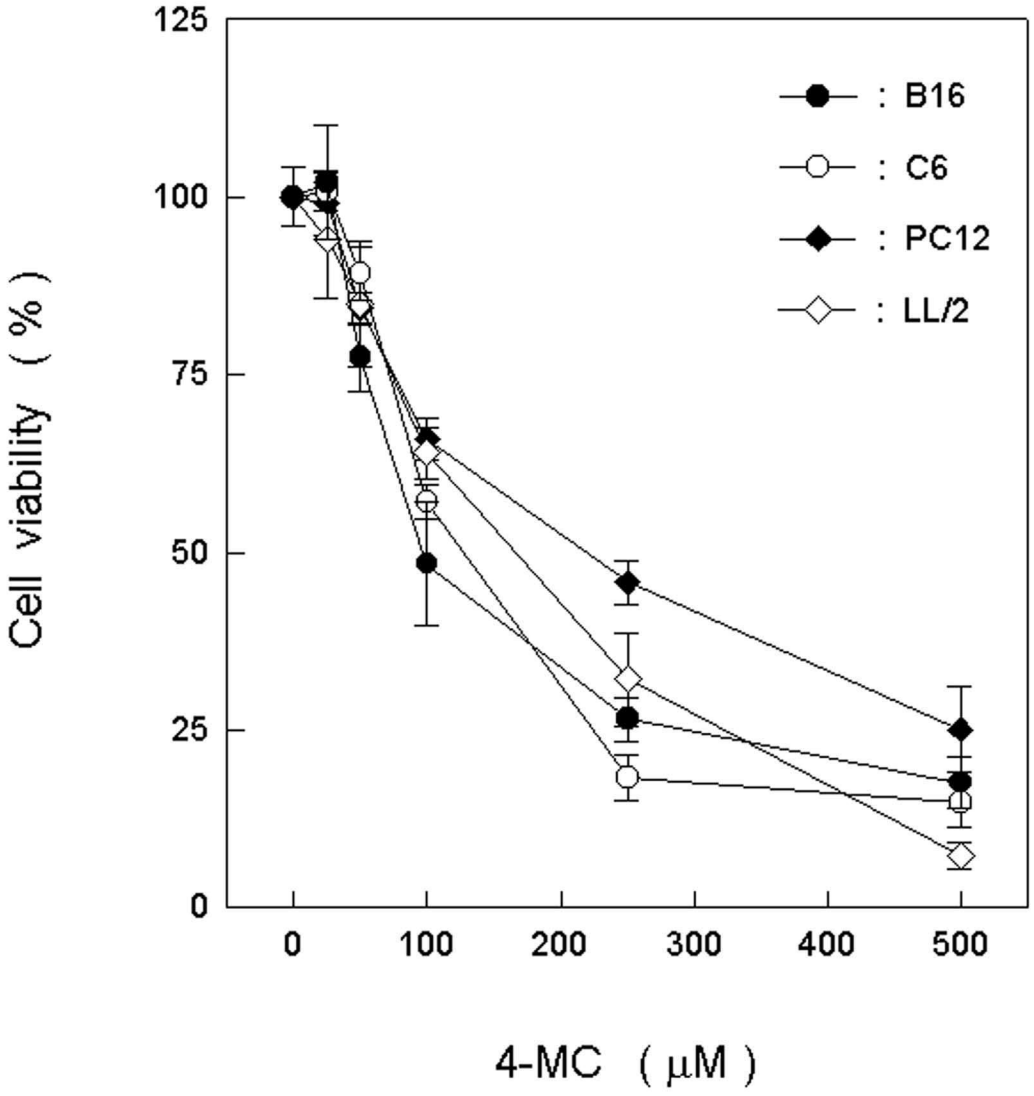

Fig. 4

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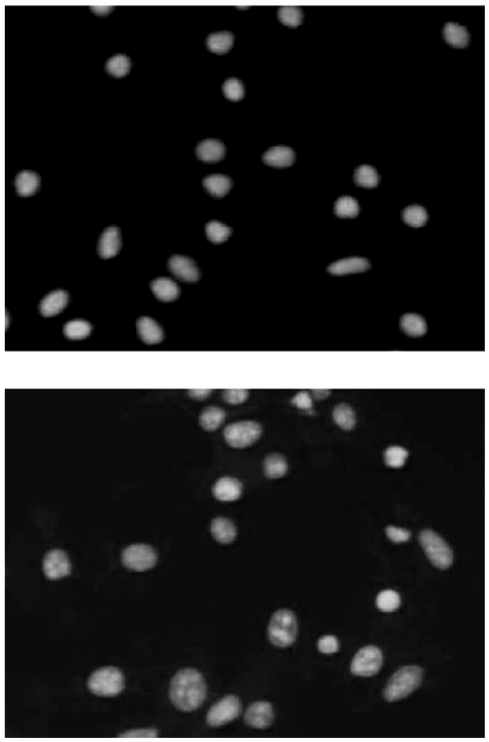
[a]



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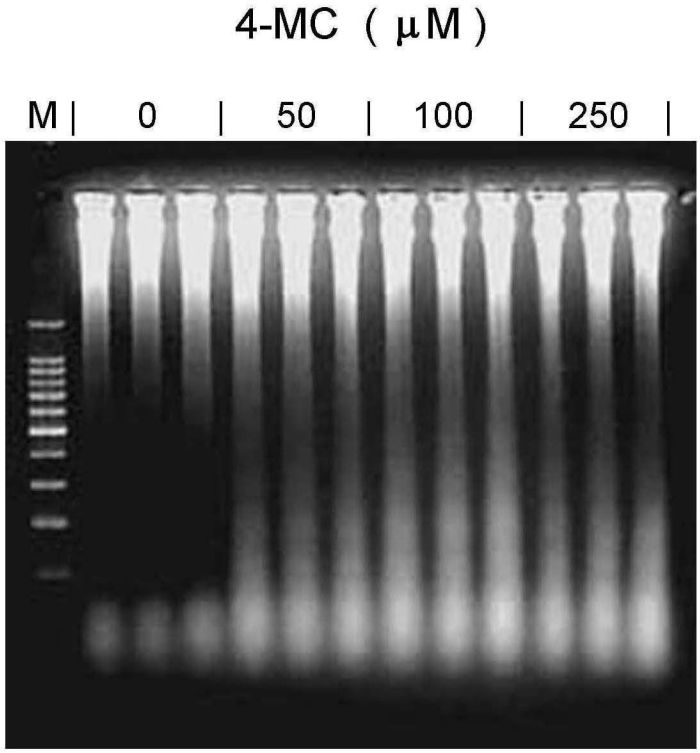

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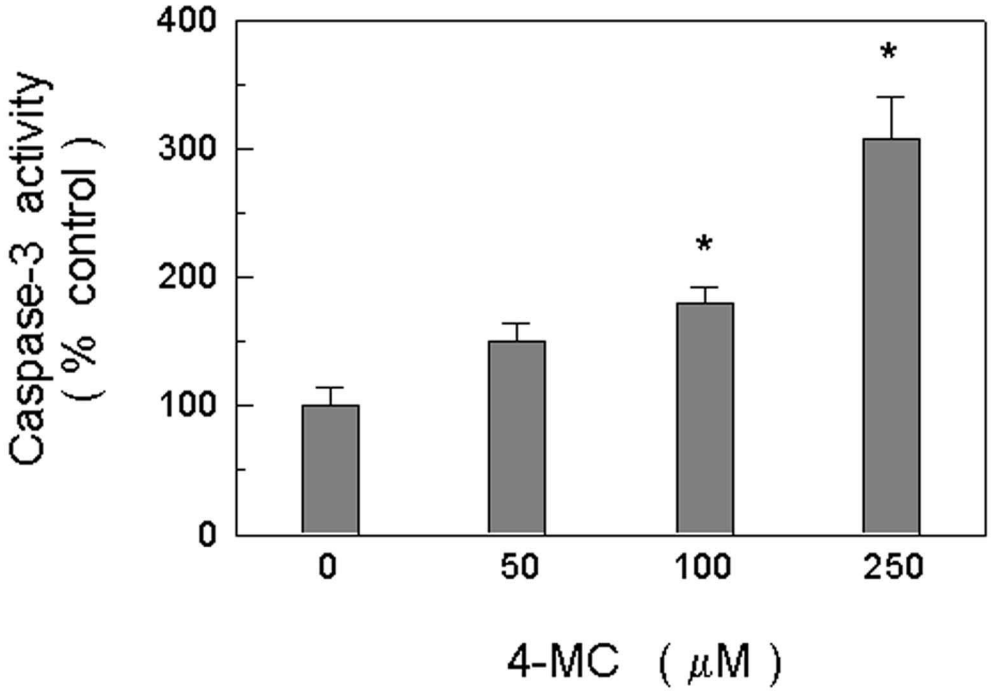

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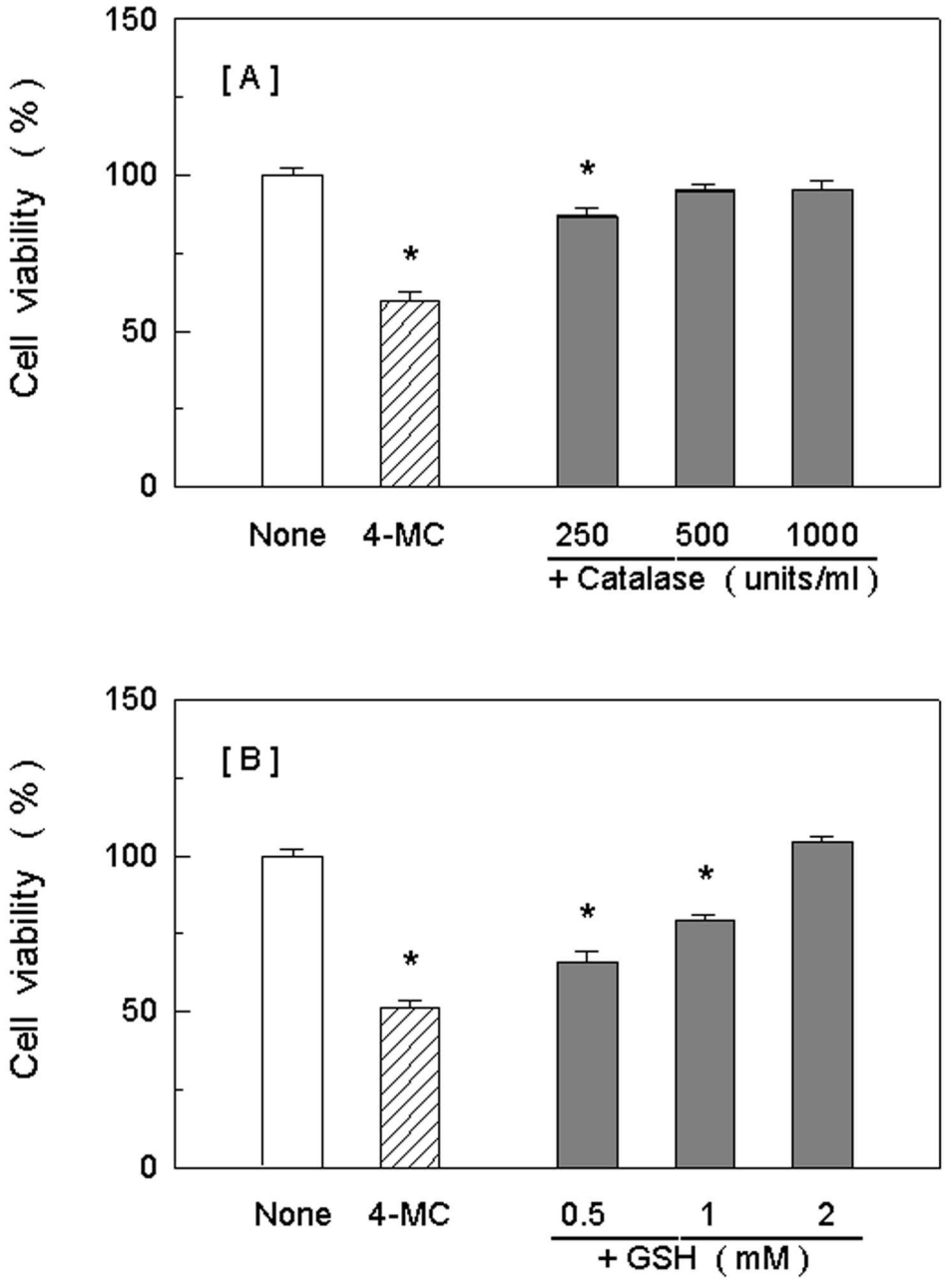

Fig. 6

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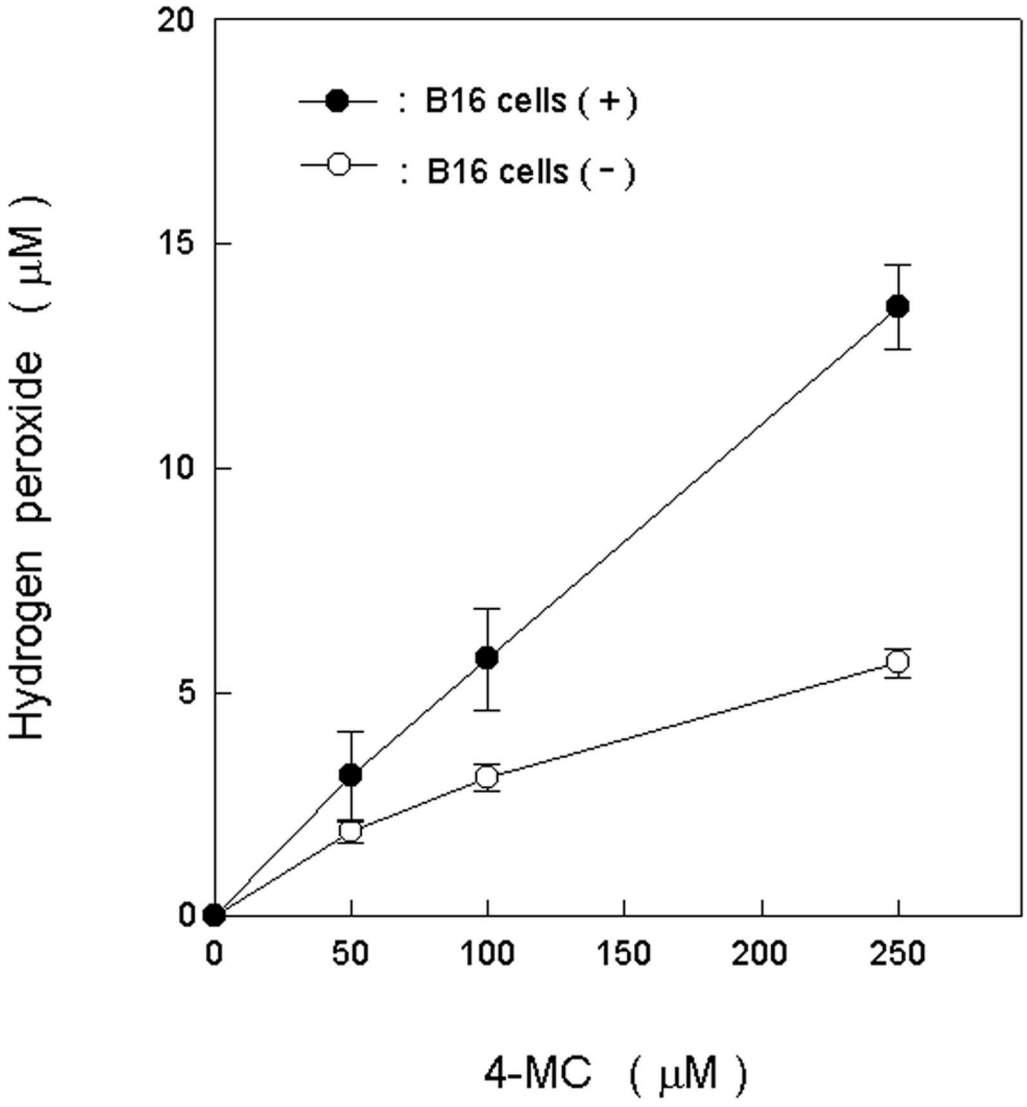

Fig. 7