— Research Letter —

Cytotoxic Characteristics of Two Isomeric Dimers Produced by Oxidation of Sesamol, an Antioxidant in Sesame Oil

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(Received May 18, 2011; Accepted June 17, 2011; Published online June 22, 2011)

Antioxidants themselves are oxidized to prevent oxidation of other molecules. One may ask if the oxidized antioxidants are safe for humans. However, there is very little information on the toxicity of oxidized antioxidants. We previously identified cytotoxic compounds in the products from oxidation of sesamol, a potent antioxidant in sesame oil. In this study using a flow cytometer with fluorescent probes, we revealed cytotoxic characteristics of two isomeric dimers (dimer A and B) in rat thymocytes. Increase in cell lethality by dimer A was more profound than those by sesamol and dimer B. The incubation of cells with dimer A increased the populations of shrunken cells and the cells with phosphatidylserine exposed on outer surface of cell membranes. Since these phenomena are parameters for early stage of apoptosis, the results indicate that dimer A may promote the process of apoptosis. However, the population of the cells containing hypodiploid DNA, a parameter for late stage of apoptosis, was decreased by the incubation with dimer A. It was not the case for dimer B. Results indicate that dimer A may inhibit the degradation of DNA during apoptosis. Taken together, it is likely that dimer A exerts both proapoptotic action and inhibitory action on late stage of apoptosis in rat thymocytes.

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Key words —— antioxidant, apoptosis, cytotoxicity, oxidation, sesamol

INTRODUCTION

Antioxidants are generally supposed to protect cells against oxidative damage, resulting in contribution to health benefits, because the compounds themselves are oxidized to prevent oxidation of biological molecules. Antioxidants are also oxidized under usual storage in air. Therefore, one may ask if the oxidized antioxidants are safe for humans. For example, the oxidized form of ascorbic acid, an antioxidant, has toxic effects on biological systems.[1] In the case of quercetin, one of naturally occurring polyphenolic antioxidants, the oxidized form of quercetin reacts with thiols and it is predicted to impair several vital enzymes.[2] However, there is still very little information on the toxicity of oxidized antioxidants.

Sesamol (3,4-methylenedioxyphenol) is one of phenolic antioxidants in sesame oil (seed oil of *Sesamum indicum*).[3,4] This antioxidant is reported to exert many beneficial actions such as antimutagenic, chemopreventive, and antihepatoxic actions.[5–10] However, we previously determined some cytotoxic compounds produced by the oxidation of sesamol.[11,12] In this study, the cytotoxic characteristics of two isomeric dimers (dimer A and B of Fig. 1) were investigated in rat thymocytes by the use of a flow cytometer with appropriate fluorescent probes. Here, we describe that one of isomeric dimers (dimer A) possesses both proapoptotic action and inhibitory action on late stage of apoptosis in rat thymocytes.

Thymocyte was used for a model experiment of cytotoxicity induced by chemical compounds[13,14] because of the following reasons. First, cell membranes remain intact because single cells are prepared without enzymatic treatment. Second, several types of hormones, biological compounds, and chemicals induce cell death (necrosis and apoptosis) in thymocytes.[15] Third, the process of cell death in thymocytes is well defined.[16]

MATERIALS AND METHODS

Isolation of Cytotoxic Dimmers —— Oxidation of

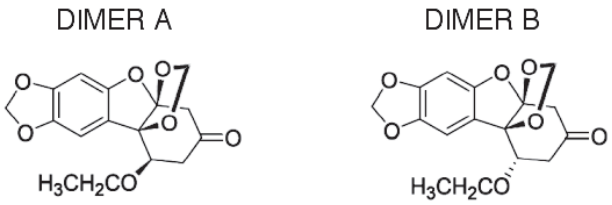

Fig. 1. Chemical Structures of Isomeric Dimers Produced by Oxidation of Sesamol

sesamol was carried out as follows.[11,12] 720 mM $FeCl_3$ aqueous solution (1 ml) was added to ethanol solution (100 ml) containing sesamol (1 g) in a screw-capped glass bottle (500 ml). The bottle was incubated for 9 days at 40°C. Thereafter, the solutions in the bottle were evaporated. The residue was dissolved in ethyl acetate, and then partitioned three times with saturated NaCl aqueous solution. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to give the oxidation mixture. The oxidation mixture was fractionated by a silica gel chromatography, eluted with ethyl acetate in hexane (20, 30, 40, 50, and 60%) to afford 10 fractions. Cytotoxic fraction was purified by a high-performance liquid chromatography and 4 major peaks (retention time, 22, 24, 30, and 35 min) were collected. Two dimers were obtained from fractions at retention times of 24 and 35 min, respectively. Structures of isomeric dimers are shown in Fig. 1.[11]

Materials and Reagents —— Propidium iodide and annexin V-fluorescein isothiocyanate (annexin V-FITC) were purchased from Molecular Probes Inc. (Eugene, OR, U.S.A.). RPMI-1640, fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), Triton X-100, and ribonuclease (RNase) A were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethylendiaminetetraacetic acid (EDTA) was supplied from Dojin Chemical Laboratory (Kumamoto, Japan). The solvent for sesamol and isomeric dimers was combination of 30% DMSO and 70% ethanol. The final concentration (0.03% DMSO and 0.07% ethanol) of solvent did not exert cytotoxic action on rat thymocytes under our *in vitro* experimental condition.

Animals, Cell Preparation, and Experimental Protocol —— This study was approved by the Committee for Animal Experiments of the University of Tokushima (No. 05279 for Oyama). The procedure to prepare cell suspension was similar to that reported previously.[13,14] In brief, thymus glands were dissected from anesthetized Wistar rats. The glands were sliced at a thickness of 0.4–0.5 mm. The slices were gently triturated in RPMI-1640 medium with glutamine (300 mg/l) and 10% FBS to enable the dissociation of lymphocytes. The RPMI-1640 medium containing the cells was passed through a mesh to remove the unwanted residue. The cell suspension was diluted to achieve approximately 5×10^5 cell/ml. The cells in 24-well Falcon tissue culture plates (2 ml in each well) were cultured at 37°C in a CO_2 incubator (Sanyo, Tokyo, Japan).

Test compounds were respectively added to cell suspensions in each well at 1 hr after a commencement of incubation, and the incubation continued for next 24 hr. The solutions containing test compounds at various millimolar concentrations were diluted to 10^3 times to achieve final micromolar concentrations.

Cytometric Analysis —— Membrane and cellular parameters were measured by the use of a flow cytometer (CytoACE-150; JASCO, Tokyo, Japan) with fluorescent probes. The parameters were similar to those described previously.[13] Cytogram (side scatter *versus* forward scatter) was routinely constructed from 2000 cells. Forward scatter intensity reflects size of each cell whereas it is cellular density of each cell in the case of side scatter intensity.

To assess cell lethality, propidium iodide was added to the cell suspension at a final concentration of 5 μM. Propidium fluorescence was measured at 2–3 min after the application of propidium iodide. The excitation wavelength for propidium was 488 nm, and the emission was detected at 600 ± 20 nm. Since propidium stains dead cells and the cells with compromised membranes, the measurement of propidium fluorescence from the cells enables an assessment of cell lethality.

To reveal the distribution of cellular DNA content, DNA was stained with the phosphate buffer solution containing 0.1% Triton X-100, 3 mM EDTA, 0.05 mg/ml RNase A, and 100 μM propidium iodide.[17] The cells were incubated with this solution for 1 hr at room temperature. Thereafter, the propidium fluorescence was measured at 600 ± 20 nm by using the flow cytometer.

The exposure of phosphatidylserine on outer thymocyte membranes, one of the parameters for early stage of apoptosis, was detected by using annexin V-FITC.[18,19] The cells were incubated with annexin V-FITC and propidium iodide for 15 min before measurement.[20,21] The excitation wavelength for the fluorescent dyes was 488 nm. The

emissions were detected at 530 ± 20 nm for FITC (annexin V binding to membranes) and 600 ± 20 nm for propidium iodide.

The activity of caspase was examined by carboxylfluorescein-carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (VAD-FMK) reagent in FAM poly caspases assay kit (Molecular Probes). This reagent enabled us to detect active caspases. The excitation wavelength for the reagent was 488 nm. The emission was detected at 530 ± 20 nm.

Statistical Analysis —— Statistical analysis was performed by Tukey's multiple comparison test. A p value of < 0.05 was considered significant. Values were expressed as mean ± standard deviation of four experiments unless otherwise described.

RESULTS

Change in Cell Lethality

The population of cells exerting propidium fluorescence (dead cells) was increased by the 24 hr incubation with 100 μM dimer A (Fig. 2A), indicating an increase in the population of dead cells. The lethality before the start of 24 hr incubation was 4.1 ± 0.9% (n = 15). As shown in Fig. 2B, the percentage population of dead cells in the control groups (N and S in Fig. 2B, respectively) without and with the solvent were 9.4 ± 2.3% and 9.6 ± 2.2% whereas it was 35.0 ± 3.1% after the 24 hr incubation with dimer A. The lethality slightly increased during the process of 24 hr incubation even under the control conditions. The changes in cell lethality by test compounds (30–100 μM sesamol, 10–100 μM dimer A, and 10–100 μM dimer B) are summarized in Fig. 2B. Significant changes were observed in the cases of 100 μM dimer A and 100 μM dimer B. The cytotoxic potency of dimer A was more profound than that of dimer B.

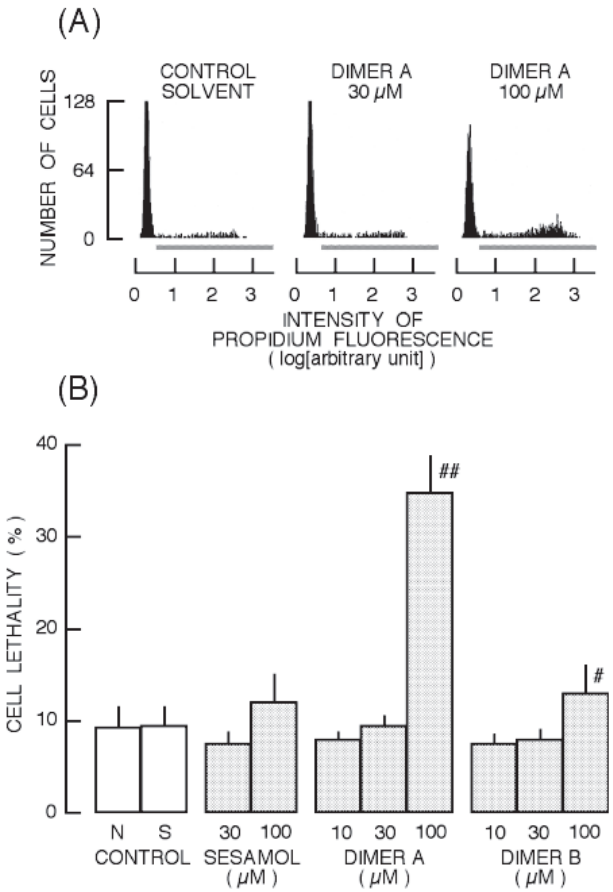

Fig. 2. Change in Cell Lethality

(A) Change in propidium fluorescence histogram by dimer A. Each histogram was constructed with 2000 cells. The line under histogram indicates the population of the cells exerting propidium fluorescence, presumably dead cells and/or the cells with compromised membranes. (B) Changes in cell lethality by sesamol, dimer A, and dimer B. Column and bar respectively show mean and standard deviation of four experiments. Symbols (# and ##) indicate significant difference ($p <$ 0.05 and $p < 0.01$, respectively) between the control groups without and with 0.1% DMSO (N and S) and the test groups.

Change in Population of Shrunken Cells

Cell shrinkage is one of the parameters for early stage of apoptosis.[22, 23] We examined the effects of isometric dimers and sesamol on forward and side scatters of rat thymocytes. As shown in Fig. 3A, the cells were mainly divided into two groups (areas NC and SC) in the cytogram. Area SC consisted of the cells with decreased intensity of forward scatter and increased intensity of side scatter, presumably shrunken cells. The 24 hr incubation of the cells with 100 μM dimer A increased the population of shrunken cells from 34.7 ± 2.4 to 81.4 ± 2.7%. The population of the shrunken cells before the start of incubation was 10.8 ± 2.0% (n = 13). Although the population spontaneously increased during the incubation without test compounds,[22, 23] further increases were observed after the 24 hr incubation with 30–100 μM dimer A and 100 μM dimer B (Fig. 3B). It was not the case for sesamol.

Change in Population of Cells Containing Hypodiploid DNA

Hypodiploid DNA is observed during late stage of apoptosis.[24–26] The incubation with dimer A increased cell lethality in association with increased population of shrunken cells (Figs. 2B and 3B). To examine the change in population of cells contain-

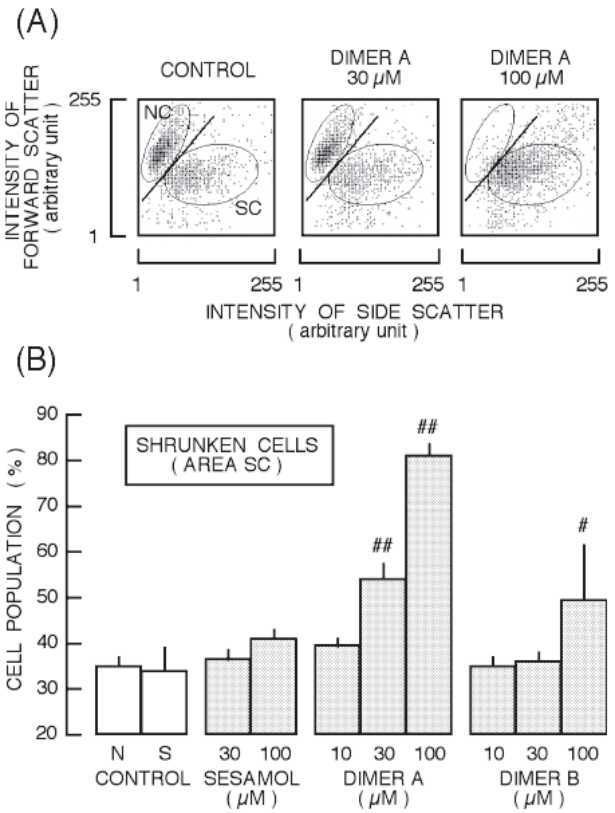

Fig. 3. Change in Population of Shrunken Cells

(A) Change in cytogram by dimer A. Each cytogram (side scatter *versus* forward scatter) was constructed with 2000 cells. Area NC consisted of normal size cells whereas shrunken cells belonged to area SC. The line between the groups was drawn by the eye measurement. (B) Changes in population of shrunken cells by sesamol and isomeric dimers. Column and bar respectively show mean and standard deviation of four experiments. Symbols (# and ##) indicate significant difference ($p < 0.05$ and $p < 0.01$, respectively) between the control groups without and with 0.1% DMSO (N and S) and the test groups.

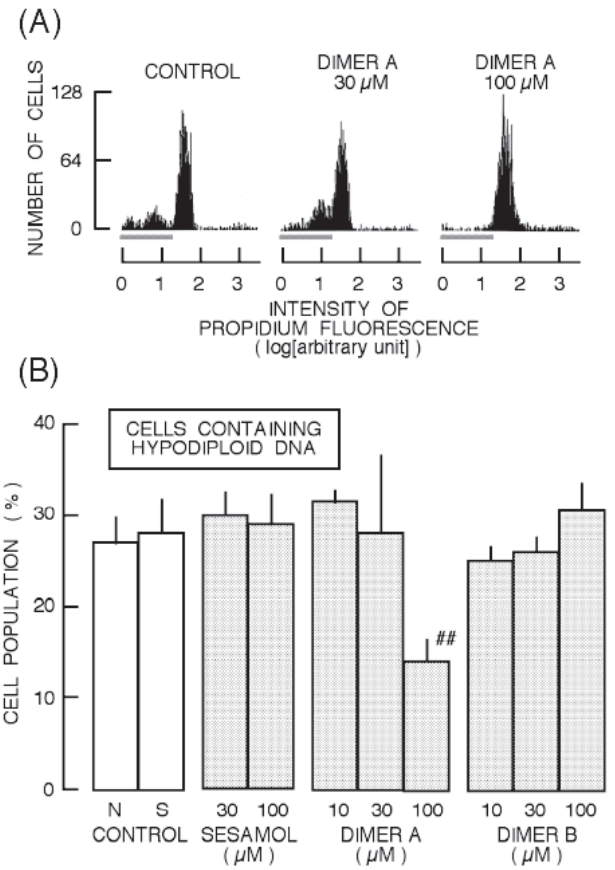

Fig. 4. Change in Population of Hypodiploid DNA Cells

(A) Change in distribution of cellular DNA content (propidium fluorescence histogram) by dimer A. Each panel was constructed with 2000 cells. The line under the histogram indicates the population of hypodiploid DNA cells. (B) Changes in population of hypodiploid DNA cells by sesamol and isomeric dimers. Column and bar respectively show mean and standard deviation of four experiments. Symbol (##) indicates significant difference ($p < 0.01$) between the control groups without and with 0.1% DMSO (N and S) and the test groups.

ing hypodiploid DNA (hypodiploid DNA cells), the distribution of cellular DNA content was examined after the 24 hr incubation with respective test compounds. As shown in Fig. 4A, the population of hypodiploid DNA cells after the incubation with 100 μM dimer A ($9.1 \pm 2.7\%$) was lower than that of control ($26.9 \pm 3.1\%$). The population of hypodiploid DNA cells before the start of incubation was $5.4 \pm 1.3\%$ ($n = 13$). Therefore, the incubation with dimer A suppressed the spontaneous increase in the population of hypodiploid DNA cells. It was not the case for dimer B.

One may argue the possibility that dimer A inhibits the activity of caspase during spontaneous apoptosis. The caspase activities of cells incubated without and with 100 μM dimer A for 6 hr were compared. The incubation for 6 hr was enough to start spontaneous apoptosis. Mean intensity of carboxyfluorescein fluorescence monitored from cells incubated with dimer A was 16.8 ± 3.2 (arbitrary unit, mean ± S.D. of three experiments) white it was 20.1 ± 2.0 in the case of cells without dimer A. The decrease in fluorescence intensity by dimer A was not significant.

Change in Population of Cells with Exposed Phosphatidylserine

The cytotoxic action of dimer A was more potent than that of dimer B (Figs. 2B–4B). Therefore, the action of dimer A was further studied. To see if dimer A increases the population of cells with phosphatidylserine exposed on outer surface of membranes, the assay using FITC-conjugated annexin V was performed.[18, 19] In the fluorescence cytogram (propidium *versus* FITC), area NF of Fig. 5A consisted of intact living cells. The 24 hr incubation

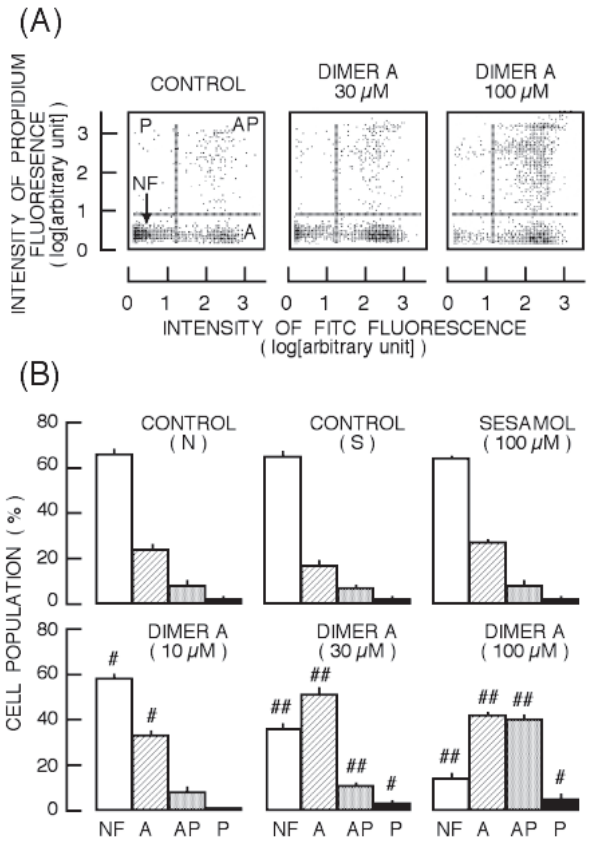

Fig. 5. Change in Groups Classified with Annexin V-FITC and Propidium Iodide

(A) Change in fluorescence cytogram (propidium fluorescence versus FITC fluorescence) by dimer A. The cells were classified by FITC and propidium fluorescences into areas NF, A, AP, and P as explained in the text. The lines between the groups were drawn by the eye measurement. (B) Changes in percentage population of areas NF, A, AP, and P by sesamol and dimer A. Column and bar respectively show mean and standard deviation of four experiments. Symbols (# and ##) indicate significant difference ($p < 0.05$ and $p < 0.01$, respectively) between control groups without and with 0.1% DMSO (N and S) and the test groups.

with 30 μM dimer A significantly decreased the population of area NF and increased both populations of cells with FITC fluorescence (apoptotic living cells, area A) and cells exerting both propidium and FITC fluorescence (apoptotic dead cells, area AP). Area P consisted of dead cells without exposed phosphatidylserine (necrotic dead cells). The population of area P was less than 5% although the incubation with dimer A increased the population (Fig. 5B). The incubation of 10 μM dimer A also decreased the population of area NF and increased that of area A without changing the population of area AP (Fig. 5B). Furthermore, significant changes in populations of areas NF and A were observed in the case of 100 μM dimer A. Results are summarized in Fig. 5B.

DISCUSSION

Actions on Apoptosis Process

The incubation with 100 μM dimer A increased the populations of shrunken cells (Fig. 3B) and the cells with phosphatidylserine exposed on the outer surface of membranes (Fig. 5B). Both phenomena are the parameters for early stage of apoptosis.[18, 19, 22, 23] However, dimer A strongly suppressed the increase in population of hypodiploid DNA cells (Fig. 4B), one of parameters for late stage of apoptosis.[24–26] Thus, the results suggest that dimer A may exert both proapoptotic action and inhibitory action on late stage of apoptosis in rat thymocytes. Although dimers A and B are isomers (Fig. 1), the cytotoxicity of dimer A was more potent than that of dimer B (Fig. 2B). The difference between the action of isomers has been generally explained by the difference in their affinity to specific molecules such as receptor and enzyme.[27] Dimer A inhibited the spontaneous increase in the population of hypodiploid DNA cells because the population increased from 5.4 ± 1.3 to $26.9 \pm 3.1\%$ during the control incubation whereas it was to $9.1 \pm 2.7\%$ in the presence of 100 μM dimer A (Fig. 4B). Thymocytes spontaneously undergo apoptosis during incubation, resulting in the increase in the population of hypodiploid DNA cells.[28] Such spontaneous increase is completely attenuated by benzyloxycarbonyl-valyl-alanyl-aspartyl-luoromethylketone (Z-VAD-FMK), a pan-inhibitor for caspases.[14, 29–31] However, dimer A did not significantly attenuate the activity of caspase. Therefore, it may be hypothesized that the process of spontaneous apoptosis is promoted by dimer A and dimer A inhibits the activity of endonuclease activated by caspases. Similar phenomenon has been reported in the case of curcumin and Jurkat cells.[32]

Implications

From many basic studies,[5–10] the antioxidant effect of sesamol, a constituent of sesame oil, is beneficial for human health. Furthermore, the antioxidant property of sesamol seems to be utilized in the treatments of hepatic and renal injuries that are induced by oxidative stress.[10, 33–36] The high resistance of sesame oil against oxidative deterioration is due to the presence of sesamol.[37, 38] However, dimer A possesses the cytotoxic action with unique characteristics on rat thymocytes (Figs. 2–5). The compounds possessing several types of cyto-

toxic actions, produced by oxidation of antioxidants, may be present in oxidized foods that contain antioxidant ingredients.

Conflict of Interest Statement All authors declare that there are no conflicts of interest in this study.

REFERENCES

1) Rose, R. C., Choi, J. L. and Bode, A. M. (1992) Short term effects of oxidized ascorbic acid on bovine corneal endothelium and human placenta. *Life Sci.*, **50**, 1543–1549.
2) Boots, A. W., Kubben, N., Haenen, G. R. M. M. and Bast, A. (2003) Oxidized quercetin reacts with thiols rather than with ascorbate: implication for quercetin supplementation. *Biochem. Biophys. Res. Commun.*, **308**, 560–565.
3) Fukuda, Y., Nagata, M., Osawa, T. and Namiki, M. (1986) Chemical aspects of the antioxidative activity of roasted sesame seed oil, and the effect of using the oil for frying. *Agric. Biol. Chem.*, **50**, 857–862.
4) Joshi, R., Kumar, M. S., Satyamoorthy, K., Unnikrisnan, M. K. and Mukherjee, T. (2005) Free radical reactions and antioxidant activities of sesamol: pulse radiolytic and biochemical studies. *J. Agric. Food Chem.*, **53**, 2696–2703.
5) Ohta, S., Suzuki, M., Sato, N., Kamogawa, A. and Shinoda, M. (1994) Protective effects of sesamol and its related compounds on carbon tetrachloride induced liver injury in rats. *Yakugaku Zasshi*, **114**, 901–910.
6) Uchida, M., Nakajin, S., Toyoshima, S. and Shinoda, M. (1996) Antioxidative effect of sesamol and related compounds on lipid peroxidation. *Biol. Pharm. Bull.*, **19**, 623–626.
7) Kaur, I. P. and Saini, A. (2000) Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity. *Mutat. Res.*, **470**, 71–76.
8) Kapadia, G. J., Azuine, M. A., Tokuda, H., Takasaki, M., Mukainaka, T., Konoshima, T. and Nishino, H. (2002) Chemopreventive effect of resveratrol, sesamol, sesame oil and sunflower oil in the Epstein-Barr virus early antigen activation assay and the mouse skin two-stage carcinogenesis. *Pharmacol. Res.*, **45**, 499–505.
9) Prasad, N. R., Menon, V. P., Vasudev, V. and Pugalendi, K. V. (2005) Radioprotective effect of sesamol on γ-radiation induced DNA damage, lipid peroxidation and antioxidants levels in cultured human lymphocytes. *Toxicology*, **209**, 225–235.
10) Hsu, D. Z., Chien, S. P., Chen, K. T. and Liu, M. Y. (2007) The effect of sesamol on systemic oxidative stress and hepatic dysfunction in acutely iron-intoxicated mice. *Shock*, **28**, 596–601.
11) Masuda, T., Shingai, Y., Fujimoto, A., Nakamura, M., Oyama, Y., Maekawa, T. and Sone, Y. (2010) Identification of cytotoxic dimers in oxidation product from sesamol, a potent antioxidant of sesame oil. *J. Agric. Food Chem.*, **58**, 10880–10885.
12) Fujimoto, A., Shingai, Y., Oyama, T. B., Kawanai, T., Hashimoto, E., Koizumi, K., Kimura, K., Masuda, T. and Oyama, Y. (2010) Apoptosis-inducing action of two products from oxidation of sesamol, an antioxidative constituent of sesame oil: a possible cytotoxicity of oxidized antioxidant. *Toxicol. In Vitro*, **24**, 1720–1726.
13) Chikahisa, L., Oyama, Y., Okazaki, E. and Noda, K. (1996) Fluorescent estimation of H_2O_2-induced changes in cell viability and cellular nonprotein thiol level of dissociated rat thymocytes. *Jpn. J. Pharmacol.*, **71**, 299–305.
14) Fujimoto, A., Sakanashi, Y., Matsui, M., Oyama, T. B., Nishimura, Y., Masuda, T. and Oyama, Y. (2009) Cytometric analysis of cytotoxicity of polyphenols and related phenolics to rat thymocytes: Potent cytotoxicity of resveratrol to normal cells. *Basic Clin. Pharmacol. Toxicol.*, **104**, 455–462.
15) Quaglino, D. and Ronchetti, I. P. (2001) Cell death in the rat thymus: A minireview. *Apoptosis*, **6**, 389–401.
16) McConkey, D. J., Jondal, M. and Orrenius, S. (1994) The regulation of apoptosis in thymocytes. *Biochem. Soc. Trans.*, **22**, 606–610.
17) Umebayashi, C., Oyama, Y., Chikahisa-Muramastu, L., Nakao, H., Nishizaki, Y., Nakata, M., Soeda, F. and Takahama, K. (2004) Tri-*n*-butyltin-induced cytotoxicity on rat thymocytes in presence and absence of serum. *Toxicol. In Vitro*, **18**, 55–61.
18) Koopman, G., Reutelingsperger, C. P., Kuijten, G. A., Keehnen, R. M., Pals, S. T. and van Oers, M. H. (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood*, **84**, 1415–1420.
19) Vermes, I., Haanen, C., Steffens-Nakken, H. and Reutelingsperger, C. (1995) A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods*, **184**, 39–51.
20) Nakata, M., Oyama, Y., Okada, Y., Chikahisa, L., Yamazaki, Y. and Satoh, M. (1999) Flow cytometric analysis on tri-n-butyltin-induced increase in annexin V binding to membranes of rat thymocytes.

Environ. Toxicol. Pharmacol., **7**, 267–273.
21) Oyama, Y., Noguchi, S., Nakata, M., Okada, Y., Yamazaki, Y., Funai, M., Chikahisa, L. and Kanemaru, K. (1999) Exposure of rat thymocytes to hydrogen peroxide increases annexin V binding to membranes: inhibitory actions of deferoxamine and quercetin. *Eur. J. Pharmacol.*, **384**, 47–52.
22) Beauvais, F., Michel, L. and Dubertret, L. (1995) Human eosinophils in culture undergo a striking and rapid shrinkage during apoptosis. Role of K^+ channels. *J. Leukoc. Biol.*, **57**, 851–855.
23) Bortner, C. D. and Cidlowski, J. A. (1998) A necessary role for cell shrinkage in apoptosis. *Biochem. Pharmacol.*, **56**, 1549–1559.
24) Nicoletti, I., Migliorati, G., Pagliacci, M. C., Grignani, F. and Riccardi, C. (1991) A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J. Immunol. Methods*, **139**, 271–279.
25) Sun, X. M., Dinsdale, D., Snowden, R. T., Cohen, G. M. and Skilleter, D. N. (1992) Characterization of apoptosis in thymocytes isolated from dexamethasone-treated rats. *Biochem. Pharmacol.*, **44**, 2131–2137.
26) Machaca, K. and Compton, M. M. (1993) Analysis of thymic lymphocyte apoptosis using in vitro techniques. *Dev. Comp. Immunol.*, **17**, 263–276.
27) Ariëns, E. J. (1986) Stereochemistry: A source of problems in medicinal chemistry. *Med. Res. Rev.*, **6**, 451–466.
28) Rinner, I., Felsner, P., Hofer, D., Globerson, A. and Schauenstein, K. (1996) Characterization of the spontaneous apoptosis of rat thymocytes in vitro. *Int. Arch. Allergy Immunol.*, **111**, 230–237.
29) Nakao, H., Umebayashi, C., Nakata, M., Nishizaki, Y., Noda, K., Okano, Y. and Oyama, Y. (2003) Formaldehyde-induced shrinkage of rat thymocytes. *J. Pharmacol. Sci.*, **91**, 83–86.
30) Iwase, K., Tatsuishi, T., Nishimura, Y., Yamaguchi, J. Y., Oyama, Y., Miyoshi, N. and Wada, M. (2004) Cytometric analysis of adverse action of diphenyl ditelluride on rat thymocytes: Cell shrinkage as a cytotoxic parameter. *Environ. Toxicol.*, **19**, 614–619.
31) Nishimura, Y., Kanada, A., Yamaguchi, J. Y., Horimoto, K., Kobayashi, M., Tatsuishi, T., Kanemaru, K., Ueno, S. Y. and Oyama, Y. (2006) Cytometric analysis of lidocaine-induced cytotoxicity: a model experiment using rat thymocytes. *Toxicology*, **218**, 48–57.
32) Sikora, E., Bielak-Zmijewska, A., Magalska, A., Piwocka, K., Mosieniak, G., Kalinowska, M., Widlak, P., Cymerman, I. A. and Bujnicki, J. M. (2006) Curcumin induces caspase-3-dependent apoptotic pathway but inhibits DNA fragmentation factor 40/caspase-activated DNase endonuclease in human Jurkat cells. *Mol. Cancer Ther.*, **5**, 927–934.
33) Hsu, D. Z., Wan, C. H., Hsu, H. F., Lin, Y. M. and Liu, M. Y. (2008) The prophylactic protective effect of sesamol against ferric-nitrilotriacetate-induced acute renal injury in mice. *Food Chem. Toxicol.*, **46**, 2736–2741.
34) Gupta, A., Sharma, S., Kaur, I. and Chopra, K. (2009) Renoprotective effects of sesamol in ferric nitrilotriacetate-induced oxidative renal injury in rats. *Basic Clin. Pharmacol. Toxicol.*, **104**, 316–321.
35) Kuhad, A., Sachdeva, A. K. and Chopra, K. (2009) Attenuation of renoinflammatory cascade in experimental model of diabetic nephropathy by sesamol. *J. Agric. Food Chem.*, **57**, 6123–6128.
36) Chandrasekaran, V. R., Hsu, D. Z. and Liu, M. Y. (2009) The protective effect of sesamol against mitochondrial oxidative stress and hepatic injury in acetaminophen-overdosed rats. *Shock*, **32**, 89–93.
37) Fukuda, Y. and Namiki, M. (1988) Recent studies on sesame seed and oil. *Nippon Shokuhin Kogyo Gakkaishi*, **35**, 552–562 (in Japanese).
38) Mohamed, H. M. A. and Awatif, I. I. (1998) The use of sesame oil unsaponifiable matter as a natural antioxidant. *Food Chem.*, **62**, 269–276.