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RESEARCH ARTICLE

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Rutin-protected BisGMA-induced cytotoxicity, genotoxicity, and apoptosis in macrophages through the reduction of the mitochondrial apoptotic pathway and induction of antioxidant enzymes

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Abstract

Bisphenol-A-glycidyldimethacrylate (BisGMA) is a resin monomer frequently used in dentin restorative treatments. The leakage of BisGMA monomer from BisGMA-based polymeric resins can lead to cytotoxicity in macrophages. Rutin has various beneficial bioeffects, including antioxidation and antiinflammation. In this study, we found that pretreatment of RAW264.7 macrophages with rutin-inhibited cytotoxicity induced by BisGMA in a concentration-dependent manner. BisGMA-induced apoptosis, which was detected by levels of phosphatidylserine from the internal to the external membrane and formation of sub-G1, and genotoxicity, which was detected by cytokinesis-blocked micronucleus and single-cell gel electrophoresis assays, were inhibited by rutin in a concentration-dependent manner. Rutin suppressed the BisGMA-induced activation of caspase-3 and -9 rather than caspase-8. Rutin inhibited the activation of the mitochondrial apoptotic pathway, including cytochrome C release and mitochondria disruption, after macrophages were treated with

Yin-Che Lu and Yu-Hsiang Kuan contributed equally to this work.

BisGMA. Finally, BisGMA-induced reactive oxygen species (ROS) generation and antioxidant enzyme (AOE) deactivation could be reversed by rutin. Parallel trends were observed in the elevation of AOE activation and inhibition of ROS generation, caspase-3 activity, mitochondrial apoptotic pathway activation, and genotoxicity. These results suggested that rutin suppressed BisGMA-induced cytotoxicity through genotoxicity, the mitochondrial apoptotic pathway, and relatively upstream factors, including reduction of ROS generation and induction of AOE.

KEYWORDS

antioxidant enzymes, BisGMA, macrophage, mitochondrial apoptotic pathway, rutin, toxic effects

1 | INTRODUCTION

Rutin, the chemical named 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside, also called rutoside, sophorin, and phytomelin, is a flavonol compound found abundantly in *Passiflora*, buckwheat seeds, citrus fruits, vegetables, and tea. Rutin possesses numerous pharmacological and beneficial effects including antioxidative, cytoprotective, anti-inflammatory, neuroprotective, cardioprotective, and anticarcinogenic activities.[1-6] Bisphenol A-glycidyl-methacrylate (BisGMA), which synthesizes epoxy resin from methacrylic acid and diglycidyl ether of bisphenol-A, is widely used in dentin restoration and as a bonding agent, a denture base material, a fissure sealant, and as bone cement.[7,8] The characteristics of BisGMA-based polymeric resins include modulus, less shrinkage, lower diffusivity, and water insolubility.[9] However, BisGMA monomer can be leachable from polymeric resins under long-term incubation with lipophilic or hydrophilic solvents.[9,10] Leachable BisGMA monomer causes harmful effects, including cytotoxic, inflammatory, and gastrointestinal responses through the activation of innate immunity.[11,12]

Macrophages play a critical role in the innate immune system and participate in innate defense against invading foreign pathogens through the production of antibacterial materials, including reactive oxygen species (ROS) and protease.[13,14] Invading foreign pathogen-induced macrophage activation induces overproduction of antibacterial materials, which result in peripheral tissue damage and inflammation.[13] Additionally, excessive activation of macrophage leads to cell damage, including DNA damage, apoptosis, genotoxicity, and cytotoxicity. BisGMA is one type of invading foreign material that could be reduced survival rate in macrophages.[15,16] In the previous studies have proposed that BisGMA-induced ROS generation leads to DNA damage and mitochondria disruption, which then leads to apoptosis in macrophages.[17,18] Until now, no studies have demonstrated the ameliorative effects of rutin on BisGMA-induced cytotoxic, genotoxic, and apoptotic effects in macrophages. In this study, we explored the protective effects and mechanism of rutin on RAW264.7 macrophage damage induced by BisGMA.

2 | MATERIALS AND METHODS

2.1 | Materials

Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin/fungizone were obtained from Hyclone (Logan, UT). Caspases fluorometric assay kits, antioxidant enzymes (AOEs) activities assay kit were obtained from Cayman (Ann Arbor, MI). Annexin V-FITC, propidium iodide (PI), and tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) were purchased from BioVision (San Jose, CA). FlowCellect Cytochrome C kit was purchased from EMD Millipore (Billerica, MA). True-Nuclear Transcription Factor Buffer Set and FITC anti-cytochrome c antibody were from Biolegend (San Diego, CA). Rutin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and other reagents, unless specifically stated, were purchased from Sigma-Aldrich (St. Louis, MO).

2.2 | Cell culture and treatments

Cells of the murine macrophage cell line RAW264.7 were purchased from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). RAW264.7 cells were maintained in DMEM supplemented with 1% penicillin/streptomycin/fungizone and 10% FBS in an incubator at 37°C with 5% CO_2.[19] Cells were grown at a density of 200 000-500 000 cells/mL in culture plates. After replacement, replacing the medium to phenol red- and serum-free DMEM, the cells were treated with rutin at the concentrations of 0, 3, 10, 30, or 100 μM for 30 minutes. The cells were treated with BisGMA at a concentration of 0.3 μM for 4 hours.

2.3 | Cytotoxicity assay

An MTT assay was used to analyze the cytotoxicity rate as described in the previous study.[16] After treatment and washing, the cells were incubated with 0.5 mg/mL MTT for 2 hours. After washing, the

intracellular blue formazan crystals were dissolved in 100 μL dimethyl sulfoxide. The optical density was measured using the Synergy HT Multi-Mode Microplate Reader (BioTek, Winooski, VT) at 550 nm.

2.4 | Annexin V-FITC and PI staining assay

Apoptosis and necrosis were evaluated using flowcytometry analysis of cells, which were stained with Annexin V-FITC and PI as described in the previous studies.[16] After cells reacted and stained, data acquisition and analyses were performed with Accuri C6 flowcytometry software (BD Biosciences, San Jose, CA). Annexin V-FITC and PI staining were detected as green and red fluorescence, respectively. Annexin V-FITC and PI negative (AnnexinV −/PI−), annexin V-FITC positive (including AnnexinV+/PI− and AnnexinV+/PI+), and annexin negative and PI positive (AnnexinV −/PI+) cells were regarded as survival, apoptotic, and necrotic cells, respectively.

2.5 | Sub-G1 formation analysis by PI staining

Apoptosis was evaluated through sub-G1 formation stained with PI as described in the previous research.[20] After treatment, the harvested cells were fixed in 70% ethanol at −20°C overnight. After being washed, the cells were incubated with RNase A and PI for 30 minutes at 37°C in the dark. The percentage of sub-G1 phase cells was analyzed using flowcytometry.

2.6 | Cytokinesis-block micronucleus assay

Chromosomal damage determined by the micronucleus (MN) assay was performed as described in the previous research.[16] After treatment, the cells were incubated with cytochalasin-B to block cytokinesis and then treated with 75 mM potassium chloride. After washing, the cells were cotreated with a cold fixative solution containing acetic acid and methanol (1:3). The cells were loaded onto the slides and stained with Giemsa solution. MN frequencies in 1000 binucleated cells were estimated using a light microscope.

2.7 | Single-cell gel electrophoresis (comet) assay

DNA strand break assessment by the comet assay was performed as described in the previous research.[16] After treatment, the cells were resuspended in low-melting-point agarose solution and placed on microscope slides precoated with normal-melting-point agarose. The cells were lysed with comet lysis solution (1% Triton X-100, 2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, and pH 10) for 1 hour at 4°C in the dark. After electrophoresis was conducted, slides were neutralized and stained with ethidium bromide. A fluorescence microscope (Nikon, Tokyo, Japan) with comet v.3 software (Kinetic Imaging Ltd., Liverpool, UK) was used to score at least 50 cells per sample. The results were expressed as % DNA in tail, which indicates relative fluorescence intensity of tail, and tail length, which indicate the distance from the head center to the end of the tail.

2.8 | Caspase-3, -8, and -9 activities assay

Caspase-3, -8, and -9 activities assessed by the commercial assay kits were performed as described in the previous research.[16] After treatment, the cells were lysed by lysis buffer for 1 hour at 4°C. After centrifugation, the protein concentration of supernatants was measured by the Bradford assay. The lysate was incubated with the fluorogenic substrate of caspase-3, -8, and -9, which is DEVD-AFC, IETD-AFC, and LEHD-AFC, respectively. After incubation, the sample fluorescence intensity was measured with the microplate reader, which has excitation and emission wavelengths at 400 and 505 nm.

2.9 | Mitochondrial membrane potential assay

Changes in mitochondrial membrane potential detected by the lipophilic cationic dye JC-1 were performed as described in the previous research.[21] After being subjected to treatment and centrifugation, the cells were resuspended in phosphate-buffered saline (PBS, pH = 7.4) and incubated with 10 mg/mL of JC-1 for 30 minutes at 37°C in the dark. Afterward, the cells' fluorescence intensities were detected by flowcytometry with excitation wavelengths at 488 nm and emission wavelengths at 525 and 585 nm for green and red fluorescence, respectively.

2.10 | Measurement of cytoC release

After being subjected to treatment and centrifugation, the cells were fixed and permeabilized using the True-Nuclear Transcription Factor Buffer Set. After being washed, the cells were stained with FITC anti-cytochrome C for 1 hour at room temperature. The fluorescence intensities were detected by flowcytometry with excitation and emission wavelengths at 488 and 525 nm, respectively.

2.11 | Measurement of intracellular ROS level

Intracellular ROS generation was determined by a semiquantitative DCFH-DA fluorescence assay as described in the previous research.[21] After being subjected to treatment and centrifugation, the cells were resuspended in PBS and incubated with 5 μM DCFH-DA for 30 minutes at 37°C in the dark. The fluorescence intensity of DCF fluorescence was assayed using a microplate reader with excitation and emission wavelengths at 488 and 525 nm, respectively.

2.12 | AOE activity assay

The activities of AOEs, including SOD, CAT, and GPx, were measured by commercial assay kits. The process for determining AOE activities was described in the previous research and performed in accordance with the manufacturer's instructions.[20]

2.13 | Statistical Analysis

Data were present as mean ± SD of three or more independent experiments. One-way analysis of variance followed by Bonferroni's post-hoc comparisons test was used for *P*-value calculations for multigroup comparison. Differences with a probability value of $P < .05$ were considered statistically significant for each test.

3 | RESULTS

3.1 | Effects of rutin on BisGMA-induced cytotoxicity in RAW264.7 macrophages

To study the effects of rutin on BisGMA-induced cytotoxicity in RAW264.7 macrophages, the cells were pretreated with various rutin concentrations for 30 minutes before the administration of BisGMA at a concentration of 50 μM for 24 hours. As shown in Figure 1, BisGMA-induced cytotoxicity was attenuated by rutin in a dose-dependent manner with a significant inhibitory effect beginning at 10 μM ($P < .05$).

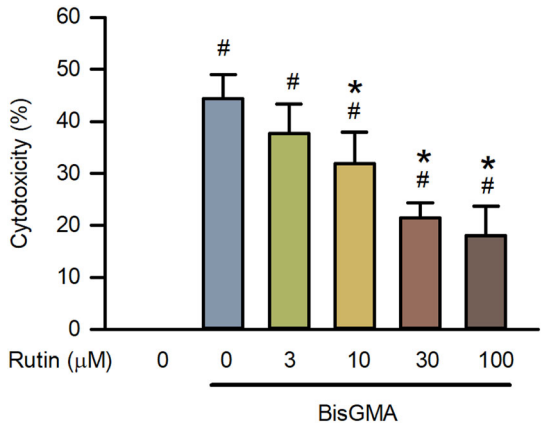

FIGURE 1 Effect of rutin on cytotoxicity in BisGMA-treated RAW264.7 macrophages. After macrophages were incubated with rutin at concentrations of 0-100 μM for 30 minutes, the cells were treated with or without BisGMA at 0.3 μM for 4 hours. Cytotoxicity was measured by an MTT colorimetric assay. Data were expressed as a percentage of the control group, which indicated treatment with BisGMA and rutin at a concentration of 0 μM. Results are expressed as means ±SD (n = 4). $^{\#}P < .05$ is considered statistically significant compared with the control group. $^{*}P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Effects of rutin on BisGMA-induced necrosis and apoptosis in RAW264.7 macrophages

After 24 hours of treatment, the partitions of necrosis and apoptosis were significantly considerably induced by BisGMA using annexin V-FITC and PI staining assay ($P < .05$). BisGMA-induced apoptosis was attenuated by rutin in a dose-dependent manner with a significant inhibitory effect beginning at 10 μM ($P < .05$). However, BisGMA-induced necrosis was not notably inhibited by rutin until the concentration at 30 μM (Figure 2A). Furthermore, we confirmed these results through sub-G1 formation analysis. As shown in Figure 2B, the formation of sub-G1 induced by BisGMA was inhibited by rutin in a dose-dependent manner with a significant inhibitory effect that began at 10 μM ($P < .05$).

3.3 | Effects of rutin on BisGMA-induced genotoxicity in RAW264.7 macrophages

MN and comet assay were used to study the protective effect of rutin on BisGMA-induced genotoxicity. MN formation was significantly induced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced MN formation in a dose-dependent manner with a significant inhibitory effect beginning at 10 μM ($P < .05$) (Figure 3A). Moreover, the tail length and %DNA in tail were significantly induced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced DNA damage in a dose-dependent manner and a significant inhibitory effect began at 10 μM ($P < .05$) (Figure 3B-D).

3.4 | Effects of rutin on BisGMA-induced caspase-3 and -9 activities in RAW264.7 macrophages

Caspase-3, -8, and -9 are key molecules in BisGMA-induced mitochondria-dependent apoptosis and genotoxicity in RAW264.7 cells.[17] Caspase-3, -8, and -9 activities were significantly induced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced caspase-3 and -9 activities in a dose-dependent manner with a significant inhibitory effect beginning at 10 μM ($P < .05$). However, BisGMA-induced caspase-8 activity was not significantly inhibited by rutin until it was at a concentration of 30 μM (Figure 4).

3.5 | Effects of rutin on BisGMA-induced cytoC release in RAW264.7 macrophages

Cytochrome C (cytoC) is released from mitochondria disruption and promotes caspase-9 activation.[22,23] CytoC liberation was significantly induced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced cytoC liberation in a dose-dependent manner with a significant inhibitory effect that began at 10 μM ($P < .05$; Figure 5).

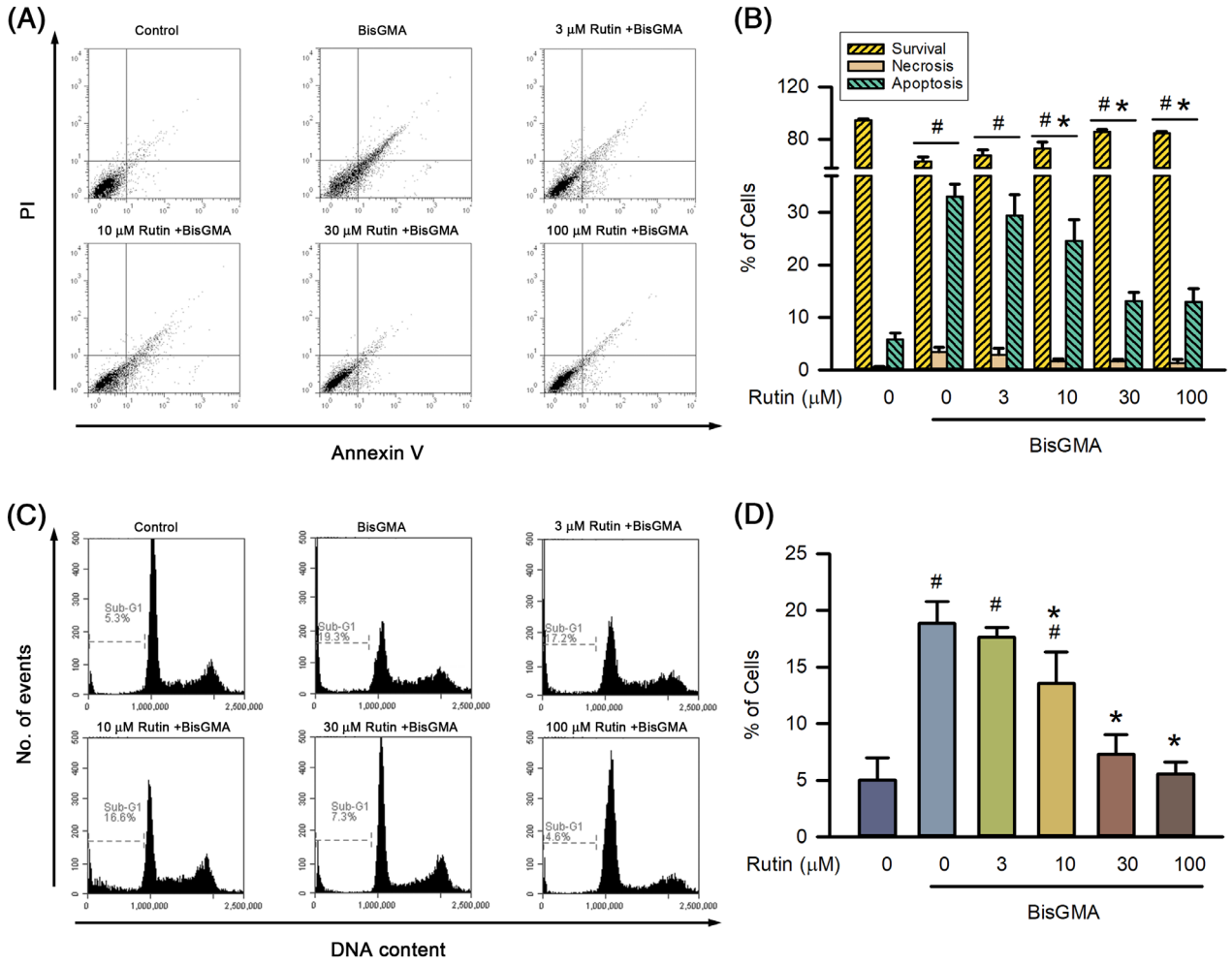

FIGURE 2 Effect of rutin on necrosis and apoptosis in BisGMA-treated RAW264.7 macrophages. After reaction and collection, the cells were stained with annexin V-FITC/PI and PI staining assay and analyzed by flowcytometry. A, The dot-plots of flowcytometry from the annexin V-FITC/PI staining assay are shown. B, After dot-plots quantitation was achieved, the percentage of necrosis, early apoptosis, late apoptosis, and viable cells was presented. C, The histograms of flow cytometry from a PI staining assay are shown. D, After histogram quantitation was conducted, the percentages of sub-G1 populations were presented. Results are expressed as means ±SD (n = 3). $^{\#}P < .05$ is considered statistically significant compared with the control group. $^{*}P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Effects of rutin on BisGMA-induced mitochondrial dysfunction in RAW264.7 macrophages

As shown in Figure 6, mitochondrial dysfunction was significantly induced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced mitochondrial dysfunction in a dose-dependent manner with a significant inhibitory effect that began at 10 μM ($P < .05$; Figure 6).

3.7 | Effects of rutin on BisGMA-induced ROS generation in RAW264.7 macrophages

Generation of ROS participates in mitochondrial dysfunction in RAW264.7 macrophages treated with BisGMA. ROS generation was significantly induced by BisGMA compared with the control group ($P < .05$). Pretreatment with rutin reduced BisGMA-induced ROS generation in a dose-dependent manner with a significant inhibitory effect that began at 10 μM ($P < .05$; Figure 7).

3.8 | Effects of rutin on BisGMA-inhibited AOE activities in RAW264.7 macrophages

For the protection of cell viability, the intracellular ROS is reduced by AOE, including GPx, SOD, and CAT. The AOE activities were significantly reduced by BisGMA as compared with the control group ($P < .05$). Pretreatment with rutin reversed BisGMA-reduced AOE activities in a dose-dependent manner with a significant reverse effect beginning at 10 μM ($P < .05$; Figure 8).

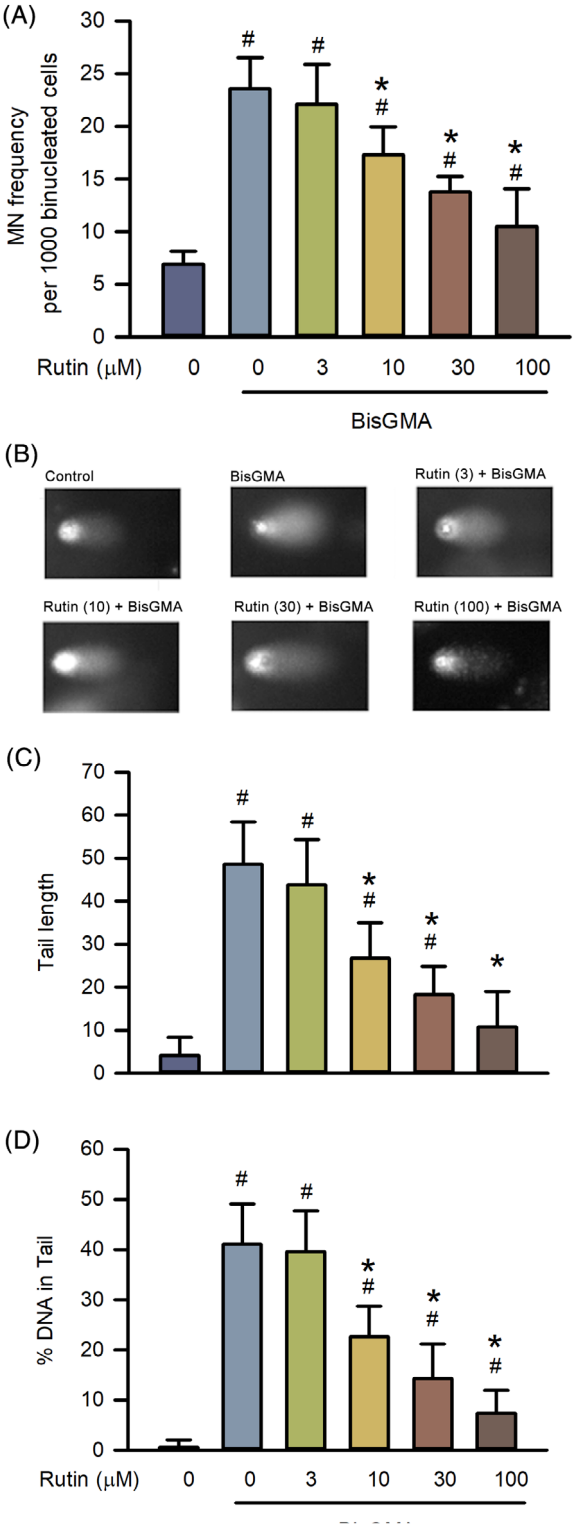

FIGURE 3 Effect of rutin on genotoxicity in BisGMA-treated RAW264.7 macrophages. A, Effect of rutin on MN formation in BisGMA-treated RAW264.7 macrophages. B, After reactions and collections were achieved, the comet assay was conducted. DNA damages were quantified as tail moment (C) and tail length (D). Results are expressed as means ±SD (n = 3). #$P < .05$ is considered statistically significant compared with the control group. *$P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

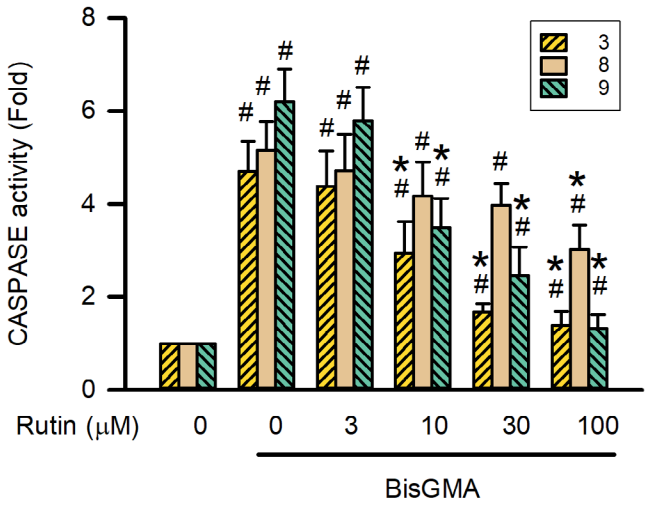

FIGURE 4 Effect of rutin on caspase activities in BisGMA-treated RAW264.7 macrophages. Results are expressed as means ±SD (n = 3). #$P < .05$ is considered statistically significant compared with the control group. *$P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Macrophages are effector cells of the nonspecific and innate immune system.[13,14] RAW264.7 cells are the murine macrophage cell line, which would not need the process of differentiation. The phenotype and functional characteristics of RAW 264.7 cell line remain stable through serial passages.[24] On the contrary, THP-1 cells and U937 cells, the human monocyte-like cell line, would be differentiated into macrophage-like cells by differentiators.[25] RAW264.7 cells are more characteristic of macrophages than THP-1 cells and U937 cells. Based on the previous studies, it has been proposed that the variability of RAW264.7 cells would be lower than the THP-1 cells and U937 cells due to the process of differentiation.[24,25] At present, we chose RAW264.7 cells for further study.

In clinical therapeutics, the polymer composite restorative materials are used in restoring the dental and bone cavities.[7,8] The most widely used monomer for the preparation of polymer composite restorative materials is BisGMA. The features of BisGMA contain high molecular weight and viscosity, low volatility, fast polymerization by organic light.[26] There is sufficient evidence to prove that BisGMA monomers dissociate from the polymer composite restorative materials after treatment with water- or organic-based solvents for 1-180 days.[9,10] Cytotoxicity could be induced by BisGMA in macrophages, dental pulp cells, and gingival fibroblasts, which can lead to oral inflammation.[11,12,17,27,28] In the previous study, it has been proposed that the treatment of RAW264.7 macrophages with 0.3 μM BisGMA for 4 hours can cause about 40% cytotoxicity.[17] At present, our results also showed that 44.37 ± 4.72% cytotoxicity induced by BisGMA at a concentration of 0.3 μM BisGMA for 4 hours in RAW264.7 macrophages. These findings were almost the same as

those reported earlier.[17] Rutin belongs to a group of plant bioflavonoid glycoside compounds called vitamin P and has a capacity for cytoprotection, antiinflammation, neuroprotection, and cardioprotection.[1-6] Based on the previous study, it has been proposed that pretreatment of SH-SY5Y neurons with 1, 5, and 10 μM of rutin for 30 minutes significantly reverses the cytotoxic effect induced by rotenone.[29] In addition, pretreatment of RAW264.7 macrophages with 40 and 80 μM of rutin significantly reverse the nitric oxide production induced by LPS.[30] At present, pretreatment with 3-100 μM of rutin for 30 minutes significantly reverse the cytotoxic effect induced by BisGMA in RAW264.7 macrophages. Rutin considerably reduced BisGMA-induced cytotoxicity in a dose-dependent manner. According to these findings, we proposed that rutin could be a potential ameliorative reagent for BisGMA-induced macrophage cytotoxicity and proinflammatory response.

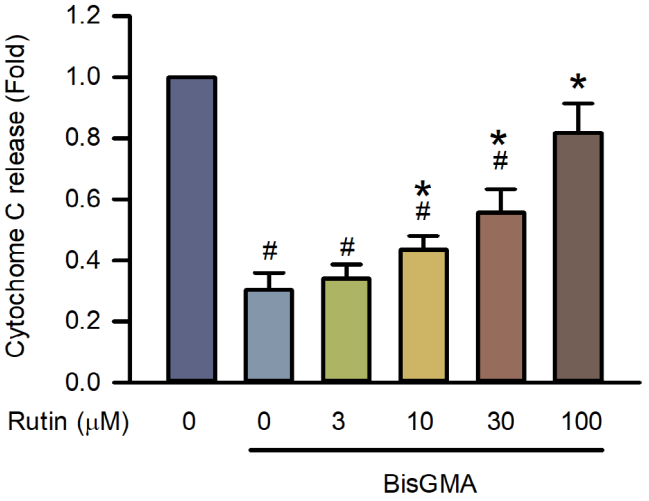

FIGURE 5 Effect of rutin on cytoC release in BisGMA-treated RAW264.7 macrophages. Results are expressed as means ±SD (n = 3). #$P < .05$ is considered statistically significant compared with the control group. *$P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

Apoptosis caused by BisGMA leads to cytotoxicity in several mammalian cells, including macrophages, dental pulp cells, and gingival fibroblasts.[17,25,31] Rutin inhibits apoptosis in the neuron of cadmium chloride ($CdCl_2$)-treated rat brain and pleural exudate cells of carrageenan-induced acute inflammation in rats.[32,33] In this study, we have demonstrated for the first time that RAW264.7 macrophages pretreated with rutin inhibit BisGMA-induced apoptosis in a dose-dependent manner through annexin V-FITC, PI staining assay, and sub-G1 formation analysis. Genotoxicity is another pathway in BisGMA-induced cytotoxicity in macrophages, gingival fibroblasts, and lymphocytes.[17,34,35] Genotoxicity and DNA damage are reduced by rutin in 2,5-hexanedione-treated rats and benzo(a)pyrene-treated mice.[36,37] Radiation-induced genotoxicity is reduced by rutin in lymphocytes.[38] Rutin administration in the present study significantly mitigated genotoxicity and DNA damage in BisGMA-incubated RAW264.7 macrophages through MN and comet assays, respectively. These results support the hypothesis that the reduction in BisGMA-induced cytotoxicity from rutin preincubation is associated with genotoxicity and apoptosis.

Under BisGMA stimulation of RAW264.7 macrophages, the caspase-dependent apoptotic pathway, which contains intrinsic and extrinsic pathways, activates caspase-3 leading to genotoxicity and apoptosis.[3,17] The proteolytic maturation of caspase-3, the

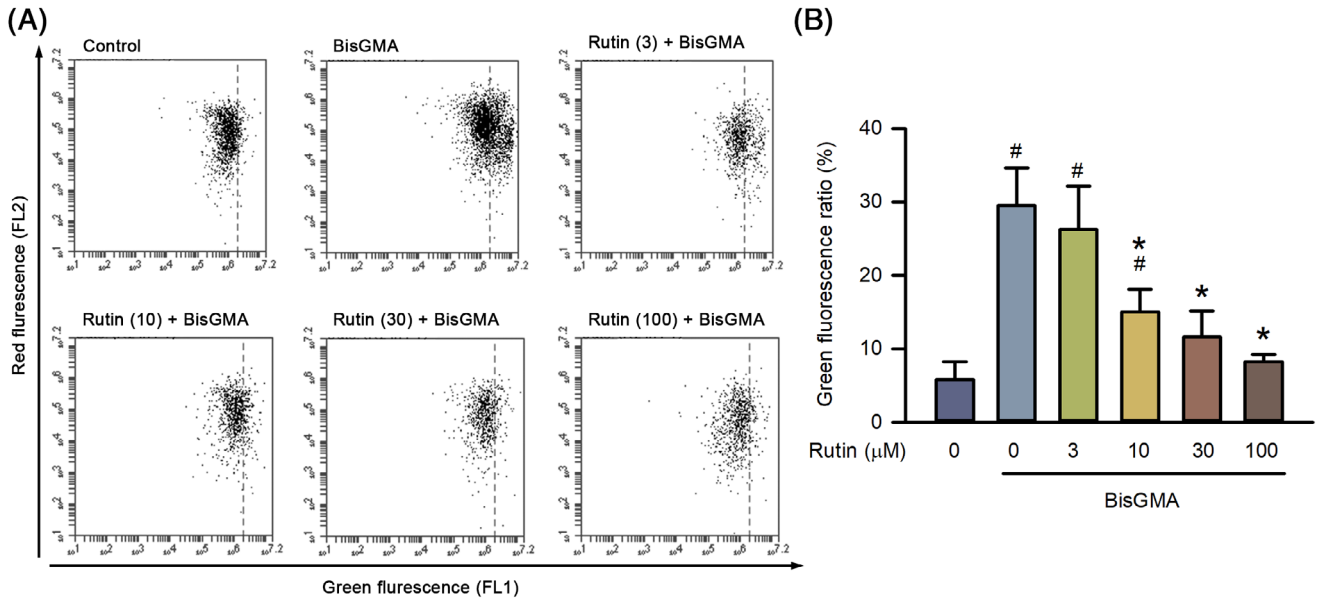

FIGURE 6 Effect of rutin on mitochondrial dysfunction in BisGMA-treated RAW264.7 macrophages. Results are expressed as means ±SD (n = 3). #$P < .05$ is considered statistically significant compared with the control group. *$P < .05$ is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

executioner caspase, is induced by the activation of caspase-8 and -9.[22] Extrinsic apoptosis can be initiated through caspase-8 activation via the formation of death-inducing signaling complex recruited by death receptor activation.[39] The intrinsic apoptosis can be initiated through caspase-9 activation via apoptosome formation, which is generated by cytoC combined with Apaf-1 in the cytosol. CytoC releases into the cytosol due to mitochondria disruption induced by an apoptotic stimulator.[22,23] Rutin inhibits apoptosis through preserving mitochondrial integrity in the brain of $CdCl_2$-treated rats and mice with traumatic brain injury.[40,41] Mitochondrial apoptosis induced by hydrogen peroxide (H_2O_2) is inhibited by rutin in human umbilical vein endothelial cells.[42] The present study first revealed that a concentration of rutin under 30 μM could not significantly strongly inhibit BisGMA-induced caspase-8 activation. By contrast, rutin inhibited BisGMA-induced caspase-3 and -9 activation in a dose-dependent manner and started a significant concentration of rutin at 10 μM. Moreover, pretreatment with rutin reduced cytoC release and mitochondria disruption in the BisGMA-treatment macrophages in a dose-dependent manner and started a significant concentration of rutin at 10 μM. Parallel trends were observed in the inhibition of caspase-3 and -9 activities, cytoC release, mitochondria disruption, apoptosis, and genotoxicity. Therefore, the reduction in BisGMA-induced apoptosis and genotoxicity from rutin pretreatment was associated with the activation of the mitochondrial apoptotic pathway.

The generation of intracellular ROS triggers the activation of the mitochondrial apoptotic pathway.[43] In H_2O_2-treated human umbilical vein endothelial cells, pretreatment rutin attenuates excessive ROS generation.[42] Rutin ameliorates carbon tetrachloride or vancomycin-induced kidney injury in mice models.[44,45] In the present study, levels of intracellular ROS could be reduced by rutin in BisGMA-treated macrophages. AOEs are capable of the deactivation and detoxification of ROS. Superoxide anion is rapidly converted by SOD into H_2O_2, which is transformed by CAT and GPx into H_2O.[46] Rutin significantly enhanced activities of SOD, CAT, and GPx in $CdCl_2$-treated rat brains, cobalt chloride-treated H9c2 myoblast cells, and endotoxin-treated injured kidneys and lungs.[6,40,47,48] We propose that pretreatment with rutin raised the activation level of SOD, CAT, and GPx in BisGMA-treated macrophages. These results indicated that rutin is able to reduce BisGMA-induced cytotoxicity and genotoxicity through the upregulation of AOE activation.

Flavonols are the most important member of secondary metabolite phytochemicals having the polyphenolic structure, which is the effective scavenger of free oxygen radicals both in vitro and in vivo.[49] The scavenging properties of oxidizing species have been found to exist on rutin, a type of flavonol.[50] Nicotinamide adenine dinucleotide phosphate oxidase (NOX)4, which is localized in mitochondria, is one of the intracellular ROS generating sources.[51] Excess ROS expression increases with NOX4 activation and results in DNA damage.[52] Rutin has been implemented in ROS-related dysfunction of the vascular endothelium and pulmonary arterial cells via NOX4 inhibition.[53,54] Based on these studies, we could hypothesize that BisGMA-induced toxic effects would be reduced by rutin via oxygen radical scavenging activity and NOX4 inhibition.

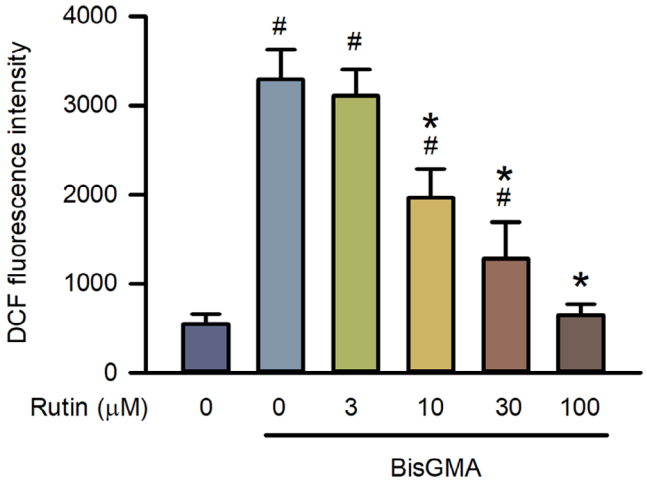

FIGURE 7 Effect of rutin on ROS generation in BisGMA-treated RAW264.7 macrophages. Results are expressed as means ±SD (n = 3). #P < .05 is considered statistically significant compared with the control group. *P < .05 is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

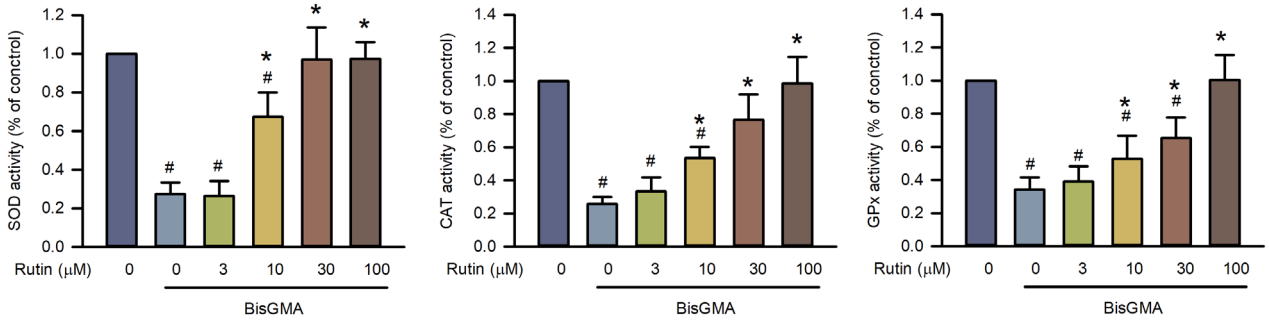

FIGURE 8 Effect of rutin on AOE activities in BisGMA-treated RAW264.7 macrophages. Results are expressed as means ±SD (n = 3). #P < .05 is considered statistically significant compared with the control group. *P < .05 is considered statistically significant compared with the BisGMA-treated group [Color figure can be viewed at wileyonlinelibrary.com]

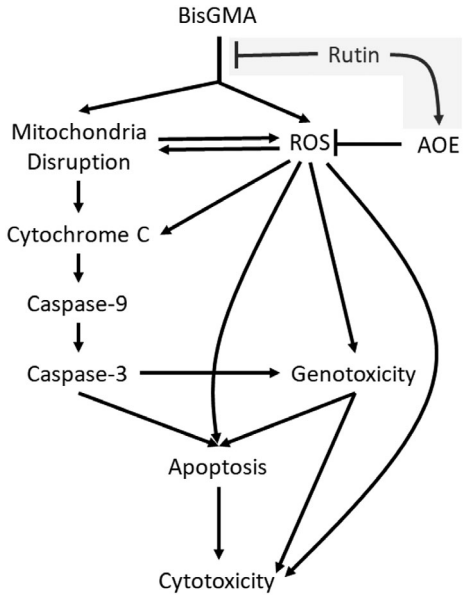

FIGURE 9 Schemes of the protective mechanisms of rutin on BisGMA-treated RAW264.7 macrophages

In conclusion, we demonstrated that macrophage cytotoxicity could be inhibited by rutin in BisGMA-treated RAW264.7 macrophages through apoptosis (Figure 9). The reduction of apoptosis was due to the inhibition of genotoxicity through reduced caspase-3 activation after treatment with rutin before BisGMA administration. BisGMA-induced caspase-3 activation was inhibited by rutin through the inhibition of caspase-8 rather than caspase-9. Moreover, rutin inhibited caspase-8 activity by downregulating the activation of mitochondrial apoptotic pathways, including cytoC release and mitochondria disruption. Finally, the protective mechanism of rutin on the BisGMA-induced activation of the mitochondrial apoptotic pathway included the diminution of ROS generation and the elevation of AOE activation. These findings show the capacity of rutin used for the reagent for the prevention of BisGMA-induced cytotoxicity, mitochondrial apoptosis, and genotoxicity in macrophages.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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