Master's Thesis

Studies on the neuroprotective activities of Citrus sunki Hort. ex Tan. against neuronal oxidative stress and neuroinflammation

Department of Medicine

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진귤 과피 에탄올 추출물의 산화적 손상과 신경염증에 대한 신경보호작용에 관한 연구

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Studies on the neuroprotective activities of Citrus sunki Hort. ex Tan. against neuronal oxidative stress and neuroinflammation

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ABSTRACT

Flavonoid compounds from citrus fruit peel have been reported to have either anti-inflammatory or anti-oxidative effects in several types of cells. In this study, we investigated the anti-inflammatory and neuroprotective effect of ethanol extract of Citrus sunki Hort. ex Tanaka peel in BV2 microglia and HT22 neurons.

It was found that ethanol extract from the fruit peel of citrus significantly suppressed several neuroinflammatory responses shown in microglia which was brain inflammatory cell. The citrus peel extract inhibited nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in LPS-stimulated microglia. It also significantly inhibited expression of pro-inflammatory cytokines such as interlukin 6 (IL-6) and suppressed the expression of the pro-inflammatory enzyme cyclooxygenase 2 (COX-2). In addition, the citrus peel extract inhibited LPS-induced phosphorylation of MAP kinase family (ERK, SAPK/JNK and P38) and decreased IκB degradation in cytosol.

On the other hand, regarding the neuroprotective activity shown in neurons, the citrus peel extract significantly increased cell viability against either glutamate-induced or H_2O_2-induced oxidative stress in HT22 neurons through the reduction of intracellular reactive oxygen species (ROS) level. The citrus peel extract was also found to inhibit oxidative stress-induced the cleavage of poly ADP-ribose polymerase (PARP) and caspase 3 activation.

Taken together, these results suggest that ethanol extract from the fruit peel of Citrus sunki Hort. ex Tanaka has neuroprotective acitivities and therapeutic potentials against neuroinflammation and oxidative stress-related neurodegenerative diseases.

Keywords: Citrus sunki Hort. ex Tanaka, neuroinflammation, neuronal oxidative stress, NF-κB, glutamate toxicity, neurodegeneration

CONTENTS

LIST OF FIGURES

Ⅰ. INTRODUCTION

Citrus fruit peel has been used in traditional Asian medicine for centuries (Huang and Wang, 1993) and in modern European herbal medicine to treat dyspepsia and related conditions (Wichtl and Bisset, 1994; Blumenthal et al., 1998). However, neuroprotective activities of citrus fruit peel has not been reported.

It has been studied that neuroinflammation and oxidative stress play a key role in many neurodegenerative diseases. Inflammation in the central nervous system (CNS) is dependent upon the synthesis of various inflammatory substances by local neurons and glia, especially resident macrophages referred to as microglia (Streit and Kincaid-Colton, 1995). Microglia are believed to play an important role in the pathway that leads to inflammation-mediated neuronal cell death in a number of neurodegenerative diseases, including Alzheimer's disease (AD; McGeer and McGeer, 2003), Parkinson's disease (PD; Kim and Joh,2006), prion diseases (Eikelenboom et al., 2002), multiple sclerosis (MS; Sanders,and De Keyser, 2007), and HIV-dementia (Boven, 2000). Activated microglia release neurotoxic and proinflammatory factors, including nitric oxide (NO), prostaglandinE2 (PGE2), and proinflammatory cytocytokines including IL-6, IL-1β, TNF-α. Uncontrolled activation of microglia may cause neuronal damage through the overproduction of proinflammatory substances, including proinflammatory cytokines, reactive proteinases, and complement proteins, in neurodegenerative diseases (Boje and Arora, 1992; Banati et al., 1993). Therefore, controlling microglial activation may have potential therapeutic options for the treatment of various neurodegenerative conditions.

Oxidative stress is also linked to various neuropathological processes, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, aging and ischemia-reperfusion injury (Li et al., 1999; Floyd, 1999; Floyd and Hensley, 2002; Lin and Beal, 2006). Oxidative stress is mediated by several agents, such as reactive oxygen species(ROS), lipid peroxidation products. Mitochondria play a central role in several pathways leading to

neuronal cell death (Cossarizza et al., 1994; Khodorov et al., 1996; Khodorov et al., 1996). Neuronal cell death is associated with Bcl family proteins to influence on mitochondrial permeability transition (MPT) pore opening, loss of mitochondrial membrane potential (MMP), and release of apoptogenic factors such as cytochrome c to the cytosol (Burke et al., 1998; Kluck et al., 1997; Burke et al., 1997). This release in turn activates caspase 9, a cysteine protease. Caspase 9 can then go on to activate caspases 3, which are responsible for destroying the cell from within.

In this study, we examined the effects of citrus peel extract on oxidative stress-induced neurotoxicity in hippocampal neurons, as well as on microglial activation. The results suggest extract from citrus fruit peel not only protected neuron cells against oxidative stress-induced neurotoxicity, but also effectively suppressed LPS-induced microglial activation. In summary, ethanol extract of citrus peel has neuroprotective acitivity and therapeutic potentials against neuroinflammation and neurodegenerative diseases via regulation of microglial activation and neuronal protection from glutamate-induced oxidative stress.

Ⅱ. MATERIALS AND METHODS

1. Ethanol extract of fruit peel from Citrus sunki Hort. ex Tanaka.

The Citrus sunki Hort. ex Tanaka peel which was isolated from the mature fruit peel of Citrus sunki Hort. ex Tanaka, is native to Korea. And it was extracted by the The Department of Life Science, Jeju National University.

2. Cell culture

HT22 cell (neuron) and BV2 cell (microglia) were maintained at 37℃ in incubator with a humidified atmosphere of 5% CO_2, and cultured in Dulecco's modified Eagle medium (DMEM, Gibco Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Gibco, Grand Island, NY, 16000-044) and 1% penicillin streptomycin.

3. Measurement of NO production

After pre-incubation of cells for 18 h, the various concentrations of compounds with LPS (200 ng/ml) were incubated for 24 h. Nitrite in culture supernatants were measured by adding 100 μl of griess reagent (1% sulfanilamide and 0.1% N-[1-naphthyl]-ethylenediamine dihydrochloride in 2.5% phosphoric acid) to 100 μl samples of medium. All measurements were performed in triplicate. The concentration of NO_2^- was calculated by comparison with a standard curve prepared using $NaNO_2$.

4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells by the TRIZOL Reagent (invitrogen, Molecular Research Center) method following the manufacturer's instructions. The RNA extraction was carried out in an RNase-free environment. The reverse transcription of 1 μg RNA was carried out using SuperScript Ⅱ Reverse Transcriptase (invitrogen), oligo (dT)15 primer, dNTP and RNase inhibitor. After incubation at 70℃ for 15 min, 42℃ for 45 min, and

transcriptase was inactivated by heating at 70℃ for 15 min. The polymerase chain reaction (PCR) was performed in a reaction buffer [cDNA 3 μl, Taq DNA polymerase 0.5 μl, 3' and 5' primer 3μl each,10 x PCR buffer 2.5 μl, dNTP Mixture 2 μl, and D.W. 11 μl, total 25 μl]. The PCR was performed with DNA gene cycler, and the amplification was followed by 30 cycles of 94 ℃ for 10min(denaturing), 55-58℃ for 45 sec (annealing) and 72℃ for 7 min (extension). The PCR products were electrophoresed on a 1% agarose gel.

5. MTT assay

The effect of citrus peel extracts on the viability of the cell was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay. Add 100 μl of MTT stock solution (2 mg/ml) into each well, after incubating for 2 h, the supernatants were aspirated. The formazan crystals in each well were dissolved in 500 μl of dimethylsulfoxide and read at A_{570} on a scanning multi-well spectrophotometer.

6. Intracellular reactive oxygen species (ROS) measurement

The DCF-DA method was used to detect the levels of intracellular ROS (Rosenkranz et al., 1992). The cells were seeded in a 96 well plate at 1×10^4 cells/well. At sixteen hours after plating, the cells were treated with citrus peel extract at 200 μg/ml and then 1 mM H_2O_2 was added to the plate. The cells were incubated for an additional 30 min at 37℃. After addition of 50 μM of DCF-DA solution for 10 min, the fluorescence of 2',7'-dichlorofluorescein was detected using a Perkin Elmer LS-5B spectrofluorometer, respectively. For image analysis for generation of intracellular ROS, after DCF-DA treatment the cells were washing with PBS, and then observed under a fluorescent microscope, which was equipped with a CoolSNAP-Pro color digital camera. For flow cytometric analasis the cells were collected and centrifuged at 1000 rpm at room temperature for 3min. Cells were washed once with PBS and subjected to the flow cytometric analysis.

7. Western blot analysis

The cells were harvested, and washed twice with PBS. The harvested cells were then lysed on ice for 30 min in 100 μl of a lysis buffer [120 mM NaCl, 40 mM Tris (pH 8), 0.1% NP 40] and centrifuged at 16,000 × g for 15 min. Supernatants were collected from the lysates and protein concentrations were determined. Aliquots of the lysates (20 μg of protein) were boiled for 15 min and electrophoresed in 10% sodium dodecysulfate-polyacrylamide gel. Blots in the gels were transferred onto nitrocellulose membranes, which were then incubated with primary antibodies. The membranes were further incubated with HRP-conjugated secondary antibodies. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK), and then exposed to X-ray film. The quantification of relative band intensities from the results was determined by Image J program.

8. Statistical analysis

All the values were represented as means ± standard error (SE). The results were subjected to an analysis of the variance (ANOVA) using the Student's- test to analyze the differences. $p<0.05$ were considered to be significant.

Ⅲ. RESULTS

1. The inhibitory effect of the citrus peel extract on LPS-induced neuroinflammation in BV2 microglia.

1.1. The cytotoxicity of the citrus peel extract was tested in BV2 microglia and HT22 neurons.

In order to determine whether citrus peel extract causes toxicity on BV2 and HT22 cells, the cell viability was tested at various concentrations of citrus peel extract in BV-2 and HT22 cells by MTT assay (Fig 1). Results showed that citrus peel extract alone at concentrations 50, 100, 200 and 400 μg/ml had no significant effect on the viability of cells. At concentrations 1 mg/ml, citrus peel extract significantly reduced the cell viability of BV2 and HT22 cells. Therefore, in this study, we used citrus peel extract at concentrations 200 μg/ml, which no effect on the cell viability for further studies of anti-inflammatory property and action mechanism in BV2 cells.

1.2. Citrus peel extract inhibited LPS-induced NO production in BV2 microglia.

To evaluate the effects of citrus peel extract on NO production in LPS-stimulated BV2 microglia, we measured nitrite released into the culture medium using the Griess reagent. BV2 microglia were treated with various concentrations of citrus peel extract (50, 100 or 200 μg/ml) for 1 h before adding LPS (200 ng/ml). The LPS-induced elevation in nitrite concentration in the medium decreased in dose-dependent manner(Fig 2).

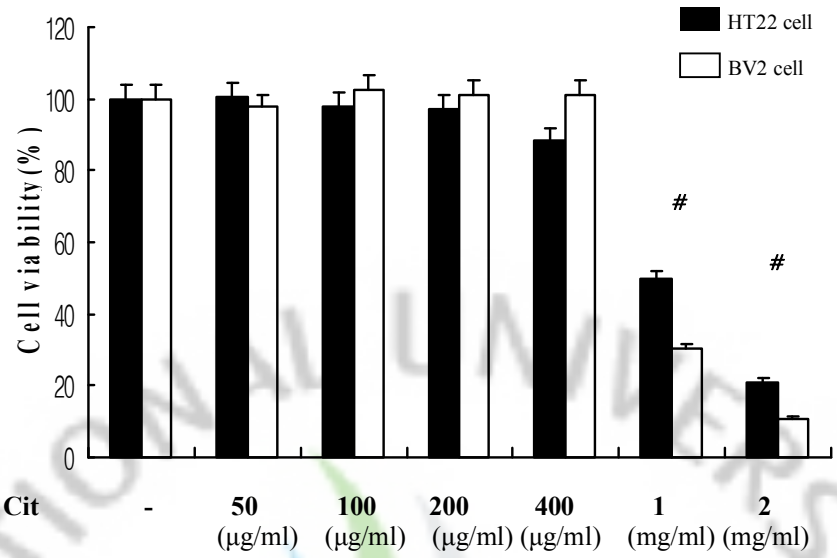


Fig.1. Dose test on cytotoxicity of the citrus peel extract in BV2 microglia and HT22 neurons. Cells were treated with citrus peel extract for 24 h at the indicated concentrations. The cell viability was determined by MTT assay. The results are mean ± SE (n=3). *P < 0.05

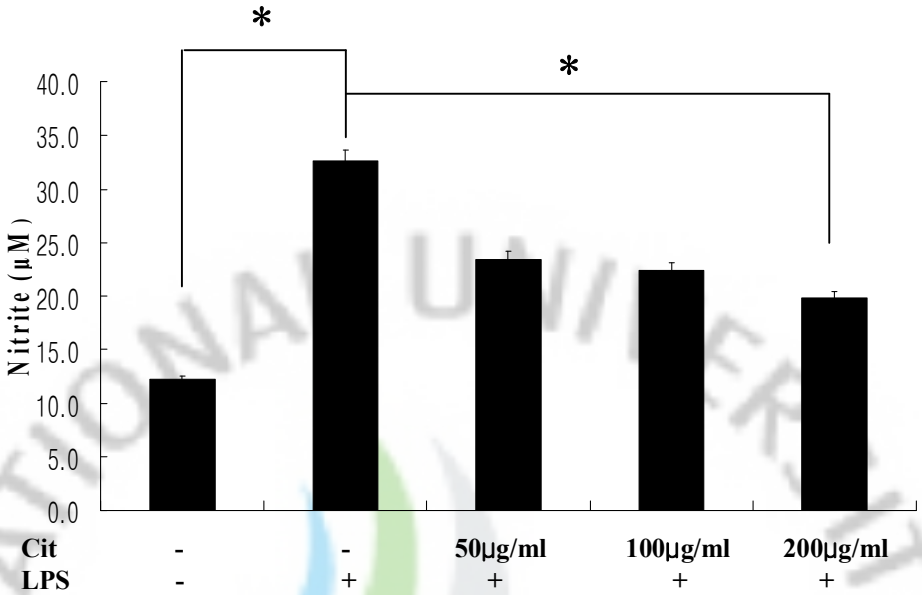


Fig.2. Inhibitory effect of citrus peel extract on LPS- stimulated NO production in BV2 microglia. BV2 cells were treated with citrus peel extract (200 μg/ml) in the presence or absence of LPS (200 ng/ml) for 24 h. Culture supernatants were then collected in order to measure NO concentrations using the Griess reaction. The results are mean ± SE (n=3). *P < 0.05

1.3. Citrus peel extract inhibited the expression of iNOS in LPS- stimulated BV2 microglia.

To investigate the mechanism by which citrus peel extract reduced LPS-induced NO production, we studied the ability of citrus peel extract to influence the LPS-induced production of iNOS. As shown by Western blot analysis, LPS treatment significantly increased the expression of iNOS. However, this expression was markedly attenuated in BV2 microglia pretreated with citrus peel extract (Fig 3).

1.4. Citrus peel extract inhibited proinflammatory cytokine production in LPS-stimulated BV2 microglia.

Next, we investigated whether citrus peel extract inhibits the production of proinflammatory cytokine IL-6 in LPS-stimulated BV-2 cells. citrus peel extract significantly inhibited the gene expression of IL-6 (Fig 4). This result suggests that citrus peel extract is effective in the inhibition of proinflammatory cytokine production through the regulation of the gene transcriptional levels of IL-6 in activated microglia.

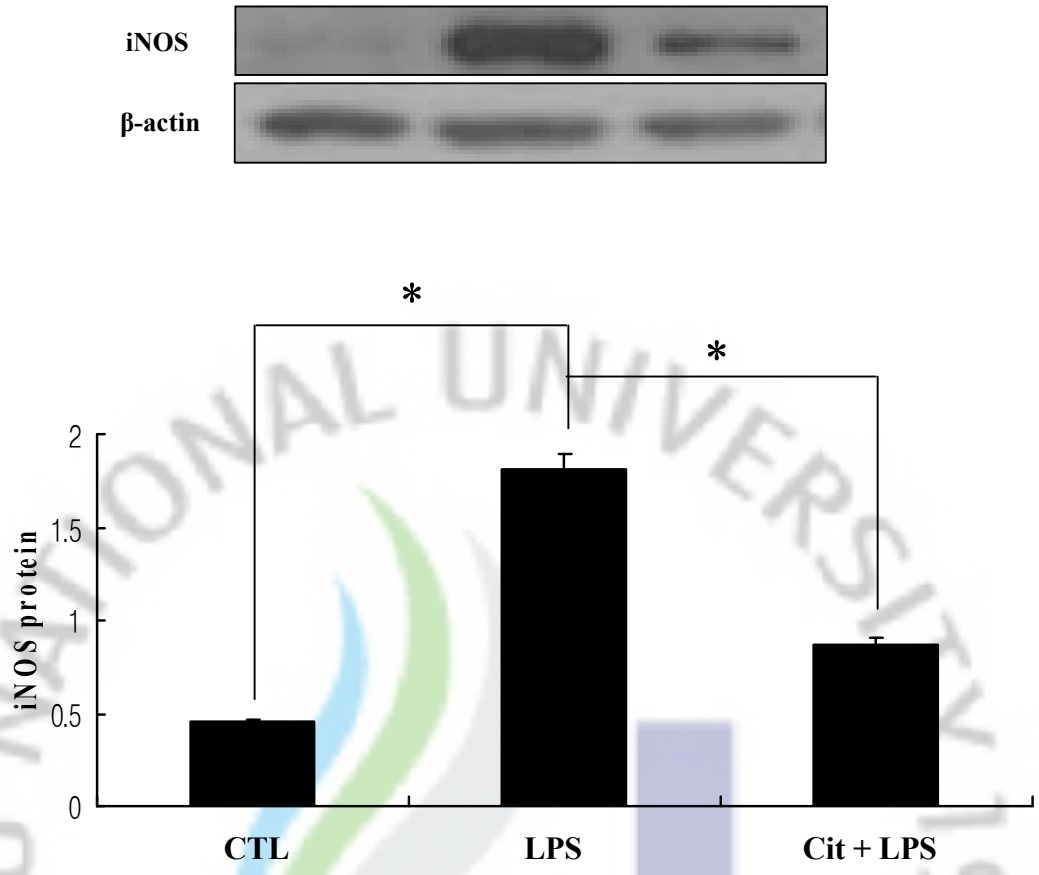


Fig.3. Inhibitory effect of citrus peel extract on the protein level of iNOS in LPS-stimulated BV2 microglia. BV2 cells were incubated with citrus peel extract in the presence or absence of LPS for 24 h. Cell lysate were extracted, and protein levels of iNOS were then analyzed by Western blotting. The quantification of relative band intensities from the results was analyzed by Image J program. The results are mean ± SE (n=3). *P < 0.05

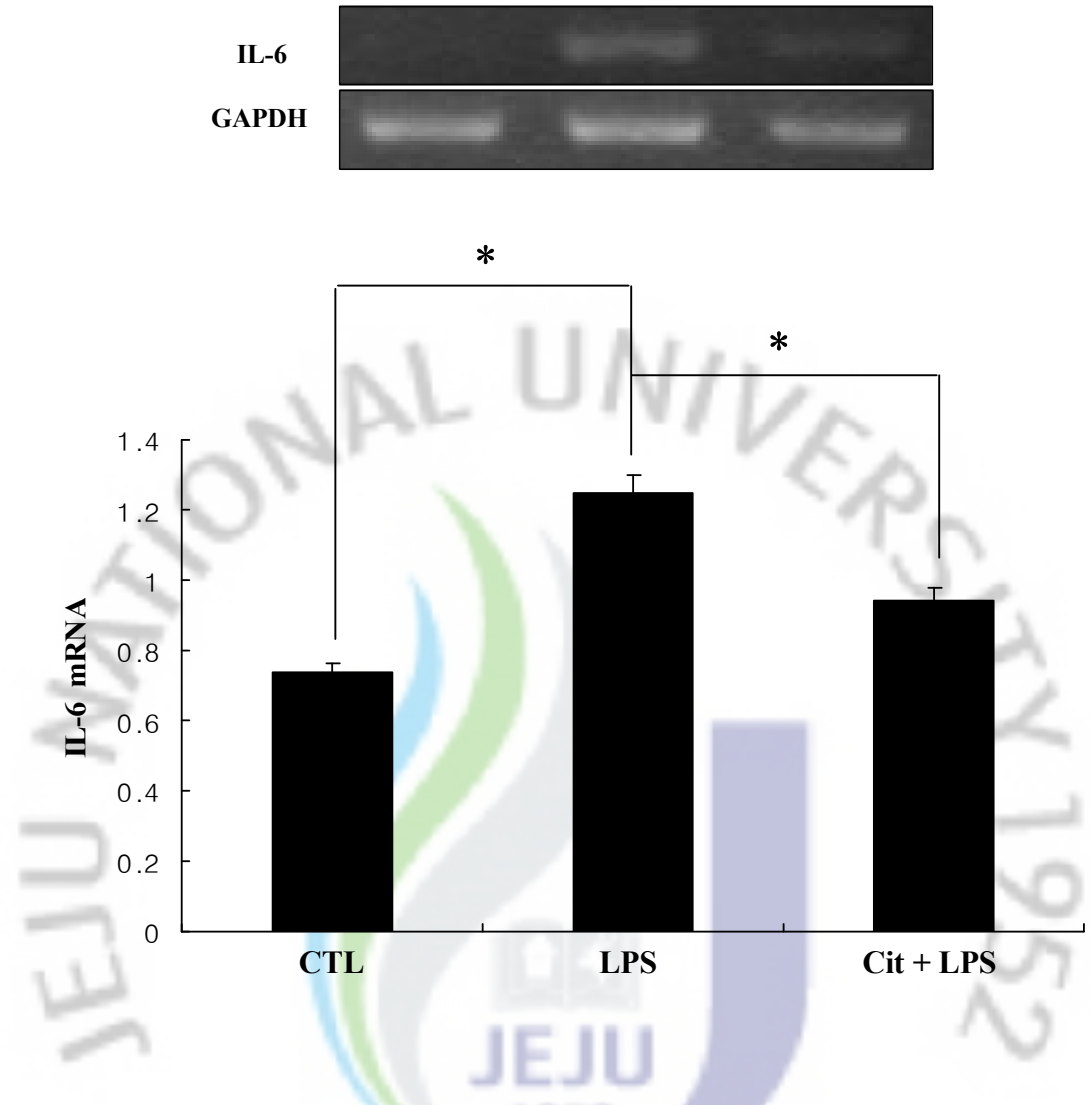


Fig.4. Inhibitory effect of citrus peel extract on the mRNA expression of IL-6 in LPS-stimulated BV2 microglia. BV2 cells were incubated with citrus peel extract for 1 h before LPS treatment, and total RNA were isolated at 24 h after LPS treatment, and the levels of IL-6 mRNAs were determined by RT-PCR. The results are mean ± SE (n=3). *P < 0.05

1.5. Citrus peel extract inhibited the expression of COX-2 in LPS-stimulated BV2 microglia.

COX-2 is the predominant cyclooxygenase at sites of inflammation. To determine the mechanism by which citrus peel extract reduced LPS-induced IL-6 production, we studied the ability of citrus peel extract to influence the LPS-induced production of COX-2. As shown by Western blot analysis, LPS treatment significantly increased the expression of COX-2. However, this expression was markedly attenuated in BV2 microglia pretreated with citrus peel extract (Fig 5). These results suggest that citrus peel extract attenuated IL-6 production through the regulation of COX-2 protein levels in activated microglia.

1.6. Citrus peel extract blocked NF-kB pathway in LPS-stimulated BV2 microglia through inhibit IκB degradation.

To investigate whether citrus peel extract acts to block the activation of the NF-kB pathway, which is implicated in the transcriptional regulation of inflammatory mediators in LPS-stimulated BV2 cells. citrus peel extract inhibited LPS-induced degradation of IκB in cytosol (Fig 6). These results indicated the potential role of NF-kB in the possible mechanism of citrus peel extract in suppressing NO, and proinflammatory cytokines in activated microglia.

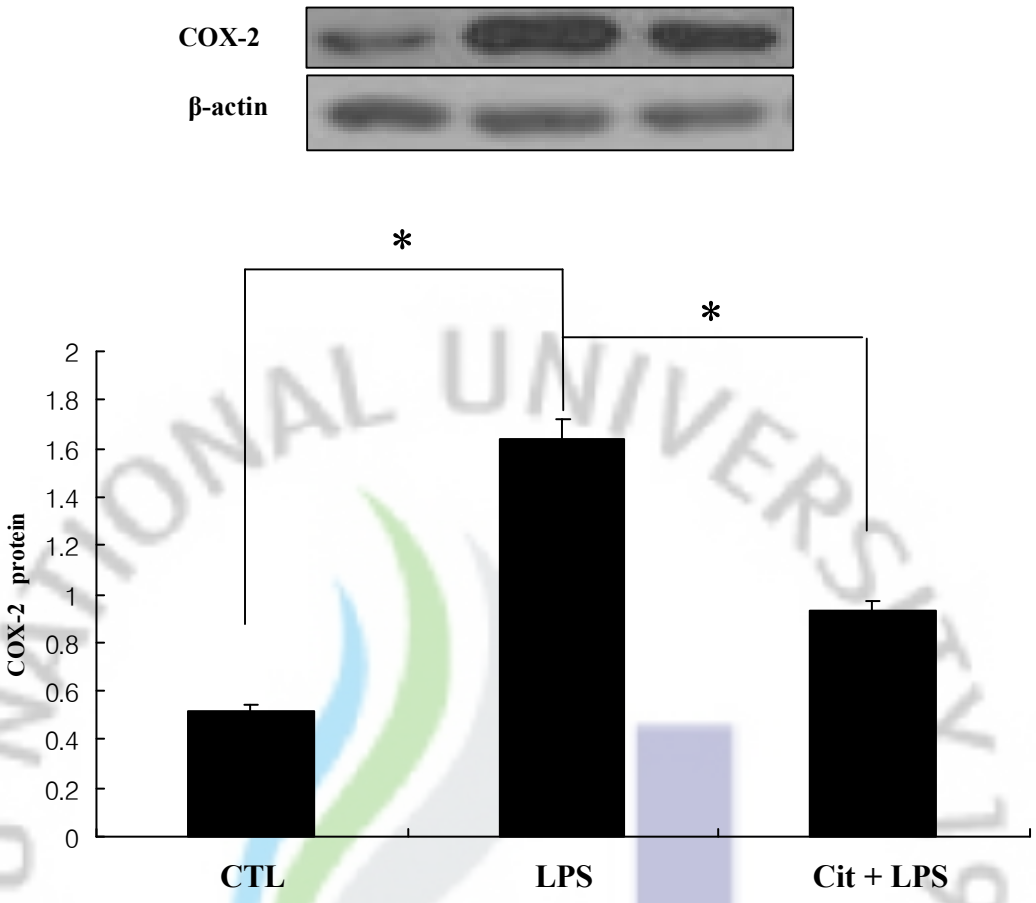


Fig.5. Inhibitory effect of citrus peel extract on the protein level of COX-2 in LPS-stimulated BV2 microglia. BV2 cells were incubated with citrus peel extract in the presence or absence of LPS for 24 h. Cell lysate were extracted, and protein levels of COX-2 were then analyzed by Western blotting. The quantification of relative band intensities from the results was analyzed by Image J program. The results are mean ± SE (n=3). *P < 0.05

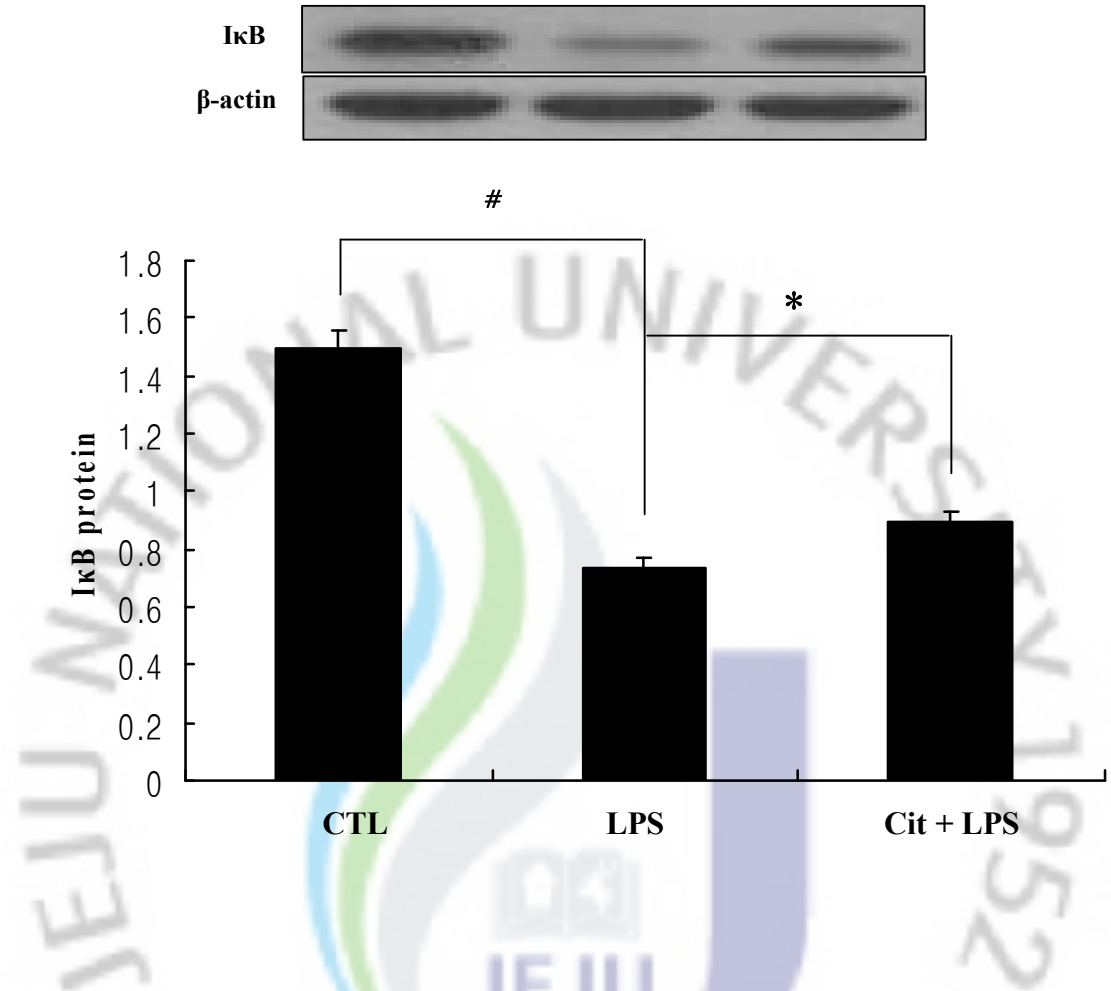
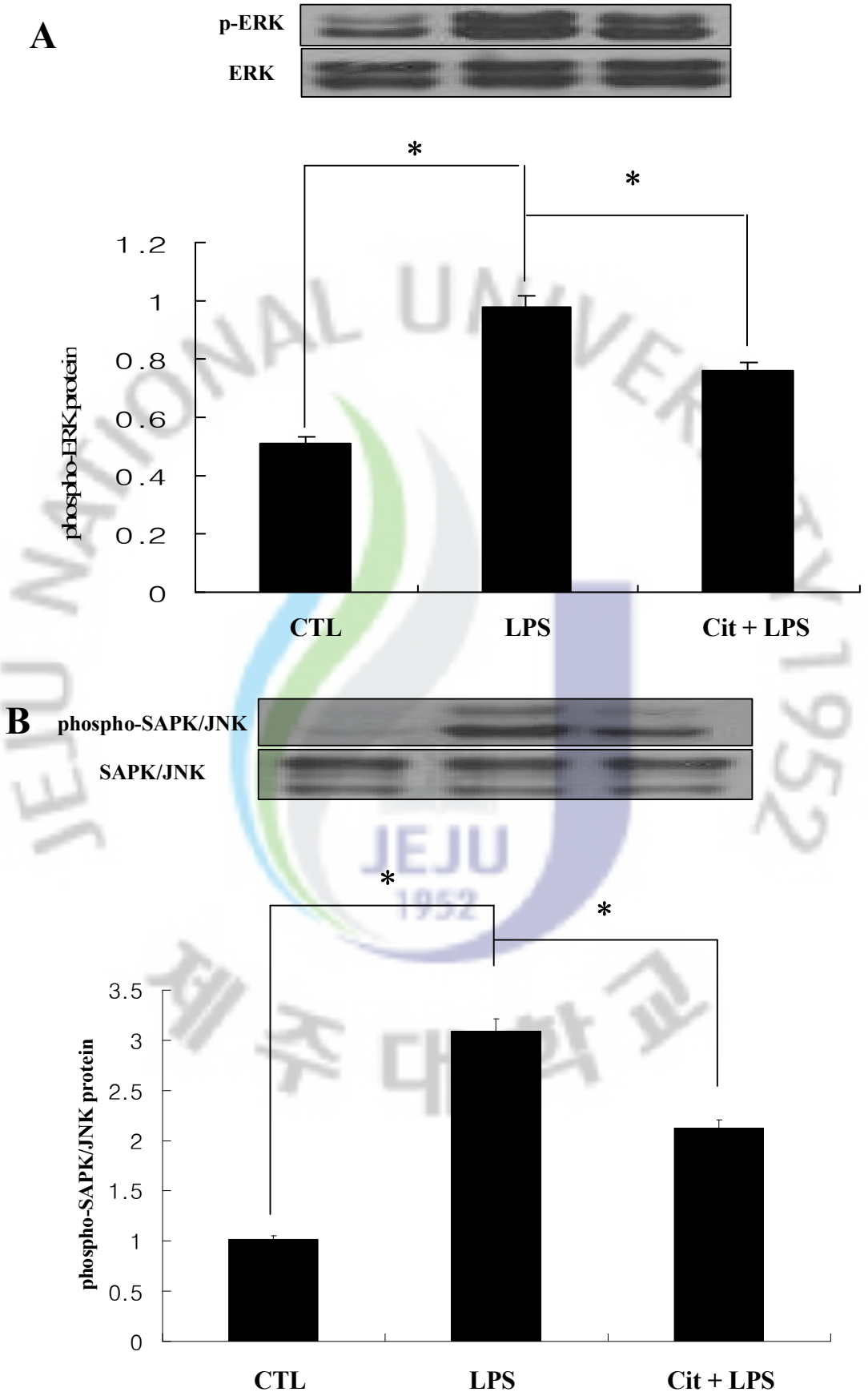


Fig.6. Inhibitory effect of citrus peel extract on the protein level of IκB in LPS-stimulated BV2 microglia. BV2 cells were treated with citrus peel extract in the presence or absence of LPS for 30 min. The cellular proteins from the cells were used for the detection of IκB by Western blotting. The quantification of relative band intensities from experimental results was determined by densitometry. The results are mean ± SE (n=3). *P < 0.05



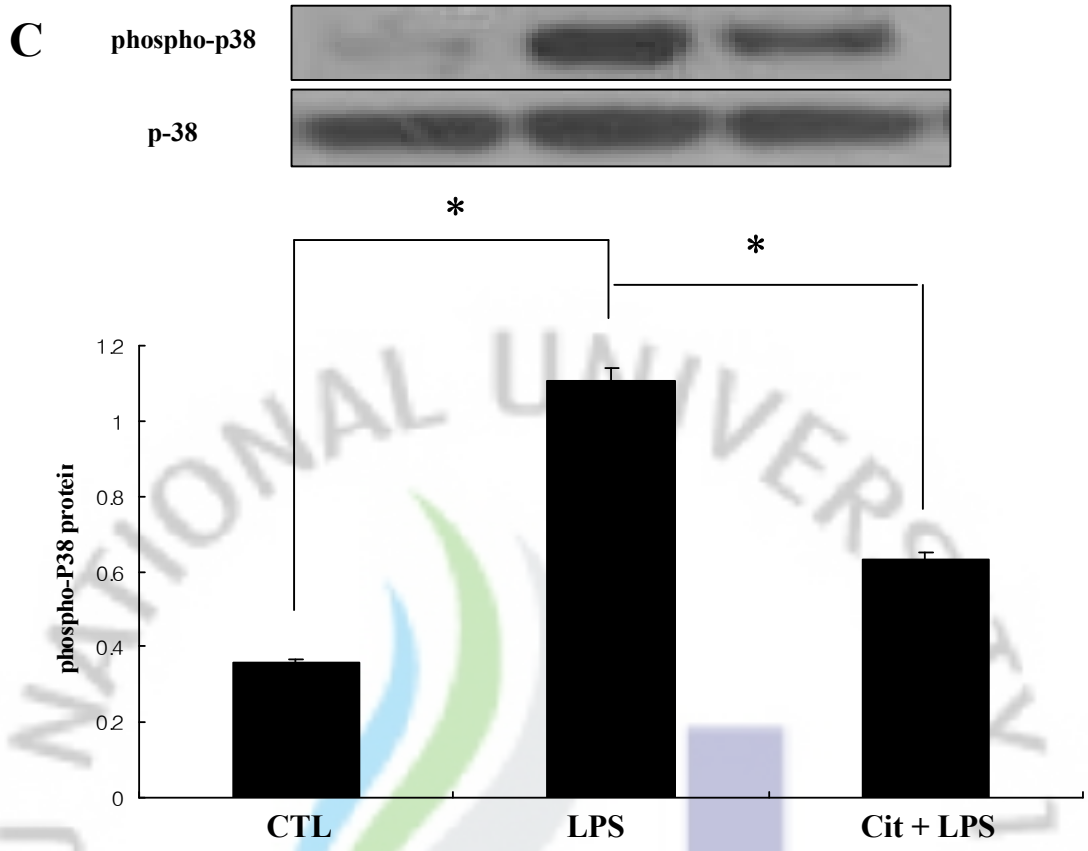


Fig.7. Inhibitory effect of citrus peel extract on the protein level of MAP kinase in LPS-stimulated BV2 microglia. BV2 cells were treated with LPS in the presence or absence of citrus peel extract for 30min. The cellular proteins from the cells were used for the detection of phosphorylated or total forms of (A) ERK1/2, (B) SAPK/JNK and (C) p38 MAPKs. The quantification of relative band intensities from the results was analyzed by Image J program. The results are mean ± SE (n=3). *P < 0.05

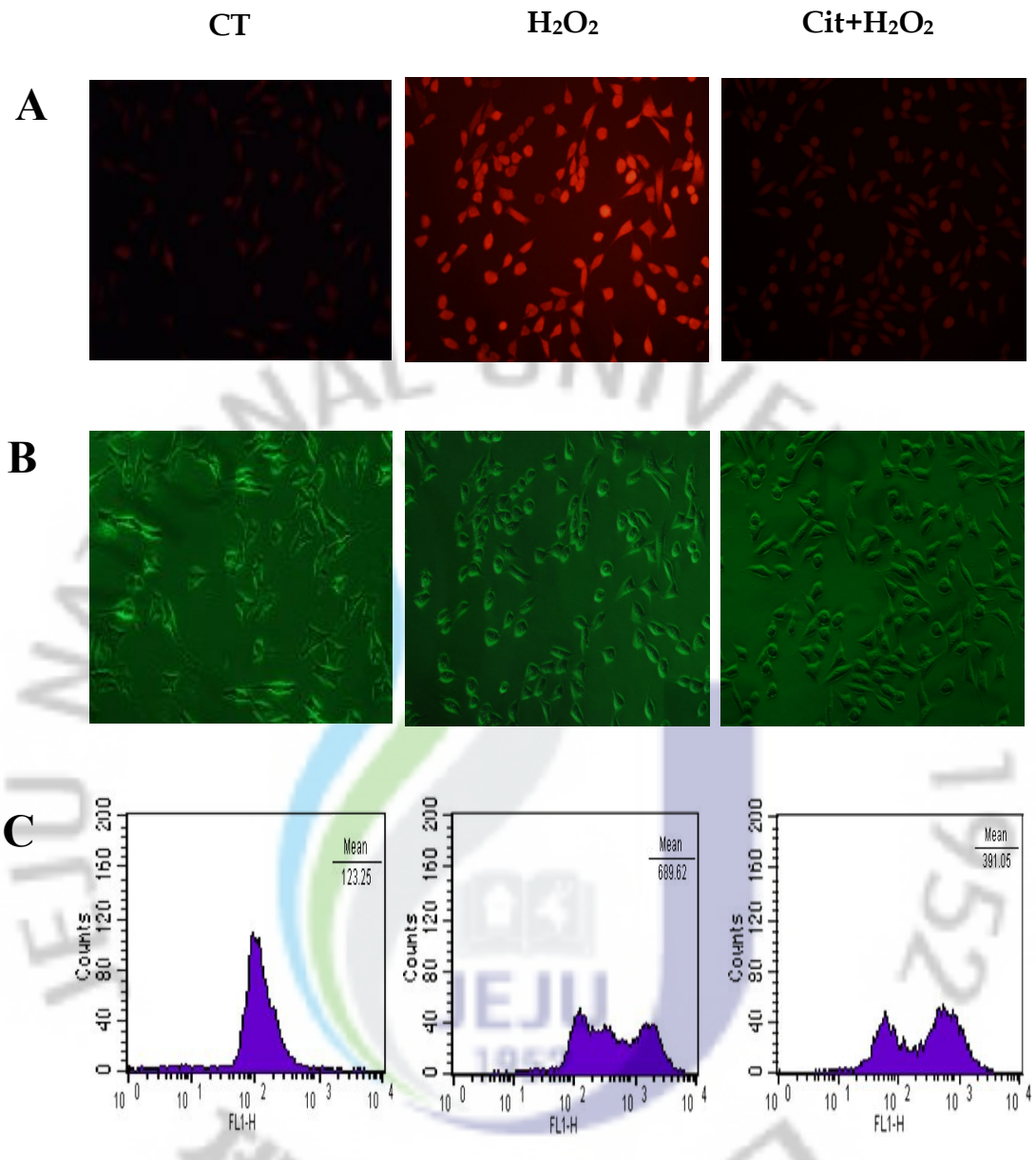
1.7. Citrus peel extract attenuated MAPK phsophorylation in LPS-stimulated BV2 microglia.

To investigate whether the MAPKs pathway was involved in the regulation of microglial inflammation, we examined the phosphorylation of three MAPK molecules, ERK1/2, SAPK/JNK, and p38 MAPK in LPS-stimulated BV2 cells. Citrus peel extract remarkably attenuated LPS-induced phosphorylation of ERK1/2, SAPK/JNK, and p38 MAPK (Fig 7), while their non-phosphorylated forms remained the same. This result indicates that signal transduction by three MAPKs might be effectively blocked by citrus peel extract in activated microglia.

2. Neuroprotective effect of citrus peel extract on oxidative stress in HT22 neurons.

2.1. Citrus peel extract reduced oxidative stress-induced intracellular ROS in HT22 neurons.

We examined the effect of citrus peel extract on the H_2O_2 or glutamate induced production of ROS in HT22 neurons. Cells were incubated with citrus peel extract in the presence or absence of H_2O_2 or glutamate. As shown in Fig 8,9 oxidative stress-stimulated HT22 neurons showed increased ROS levels. In contrast, pretreatment of cells with citrus peel extract resulted in a significant reduction of ROS. After H_2O_2 treatment, microscopic investigation shows retracted dendrites and shrunken cell bodies (Fig 8 B). However pretreatment of citrus peel extract reversed these morphological changes.



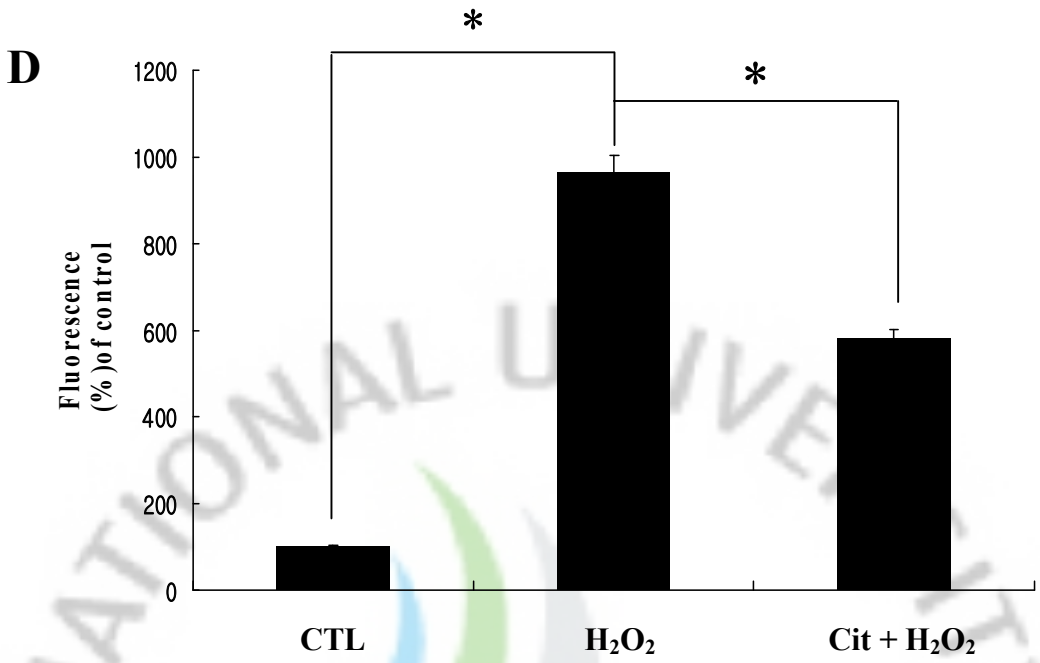


Fig.8. Neuroprotective effect of citrus peel extract on intracellular ROS level in H_2O_2 treated HT22 neurons. HT22 neurons were pretreated with the indicated concentrations of citrus peel extract before H_2O_2 treatment for 30min. The intracellular ROS level was detected by representative images were observed under a fluorescence microscope (A), microscopic visualization of HT22 neurons (B), spectrofluorometer (C), flow cytometer (D).

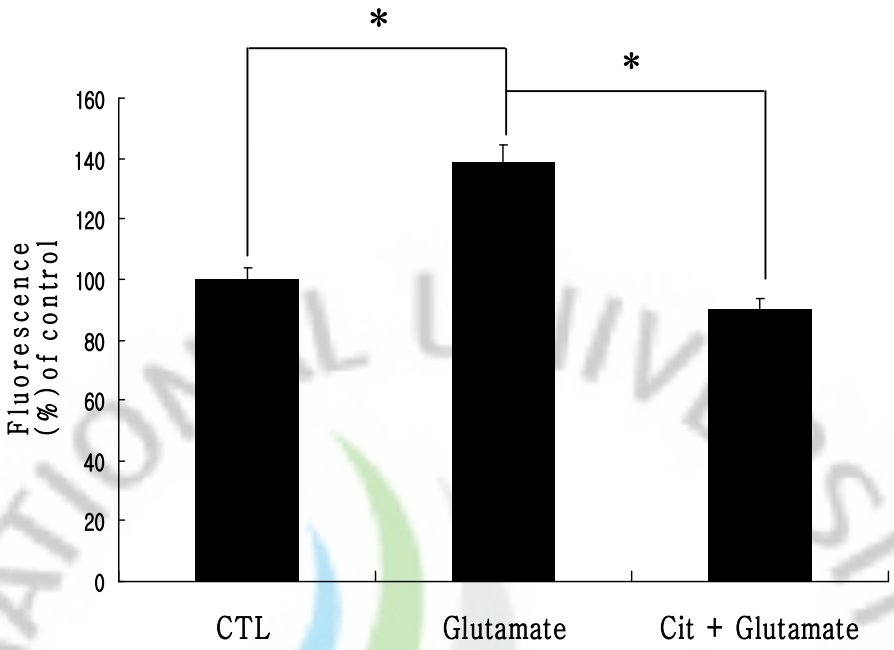


Fig.9. Neuroprotective effect of citrus peel extract on intracellular ROS generation in glutamate treated HT22 neurons. HT22 neurons were treated with gluatamte in the presence or absence of citrus peel extract for 10h. The intracellular ROS generated was detected by spectrofluorometer.

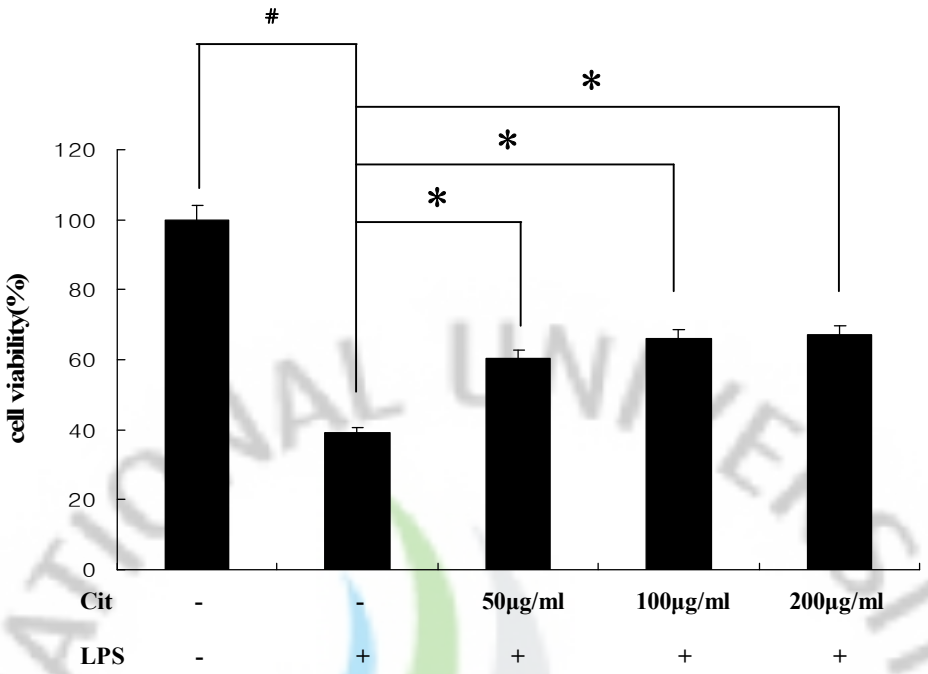


Fig.10. Neuroprotective effect of citrus peel extract on H_2O_2- induced cell death in HT22 neurons. H_2O_2 (1 mM) was added to HT22 neurons and cell viabilities were measured by MTT Assay. The viabilities of the HT22 cells treated with citrus peel extract at 50, 100 and 200 μg/ml were increased. At 200 μg/ml, the citrus peel extract significantly increased the cell viabilities. The results are mean ± SE (n=3). *P < 0.05 and # P <0.005.

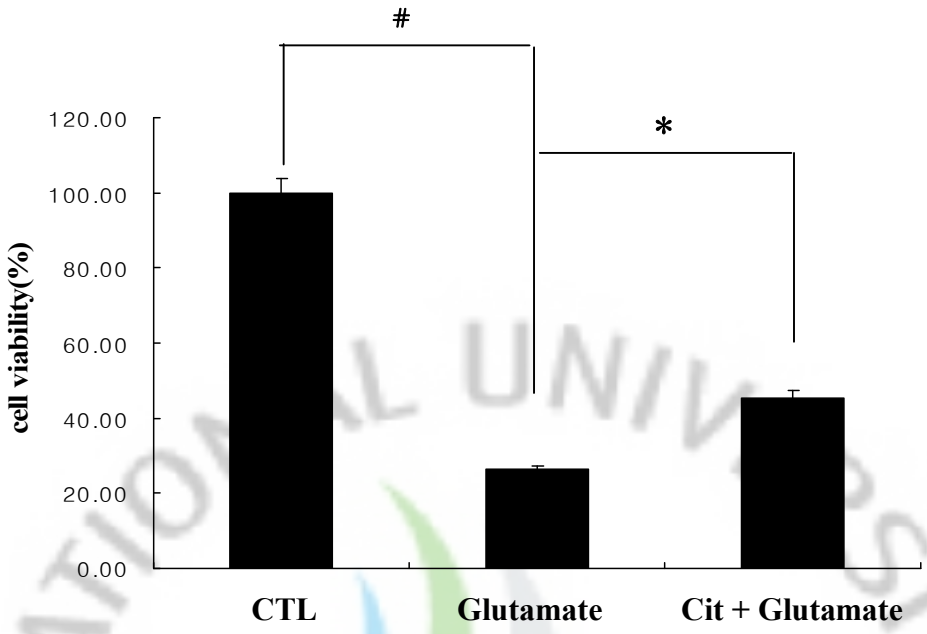


Fig.11. Neuroprotective effect of citrus peel extract on glutamate- induced cell death in HT22 neurons. The survival rate of HT22 neurons was decreased when the cells were treated with the glutamate for 24 h, compared with those cells not exposed to the glutamate treatment. At 200 μg/ml, the citrus peel extract significantly increased the cell viabilities. The results are mean ± SE (n=3). *P < 0.05 and # P <0.005.

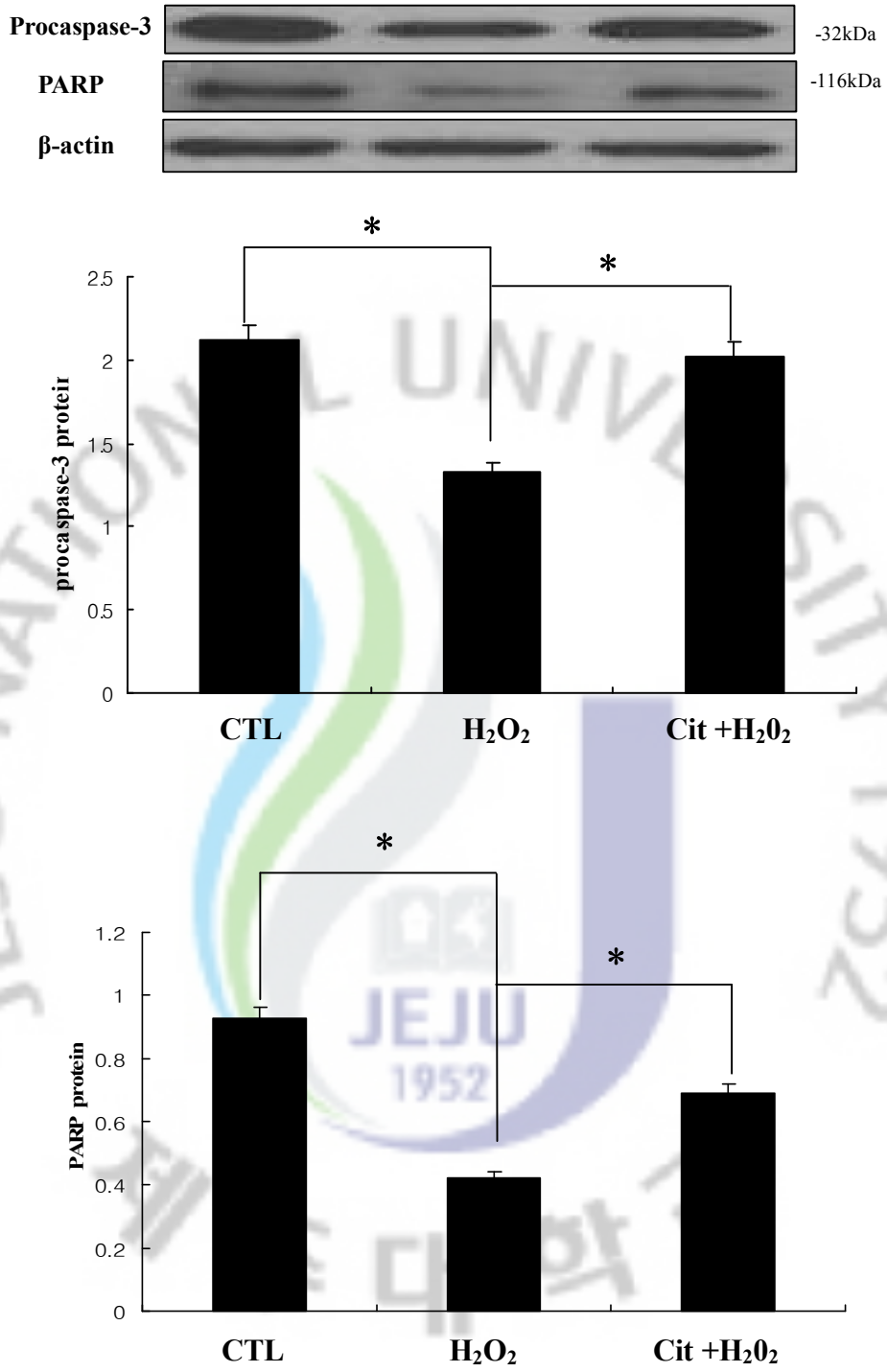


Fig.12. Effect of citrus peel extract on the protein level of procaspase-3 and PARP in on H_2O_2- treated HT22 neurons. HT22 neurons were treated with 1 mM for 10h. Levels of procaspase-3, PARP and β-actin protein were determined by western blotting. The quantification of relative band intensities from the results was analyzed by Image J program. The results are mean ± SE (n=3). *P < 0.05

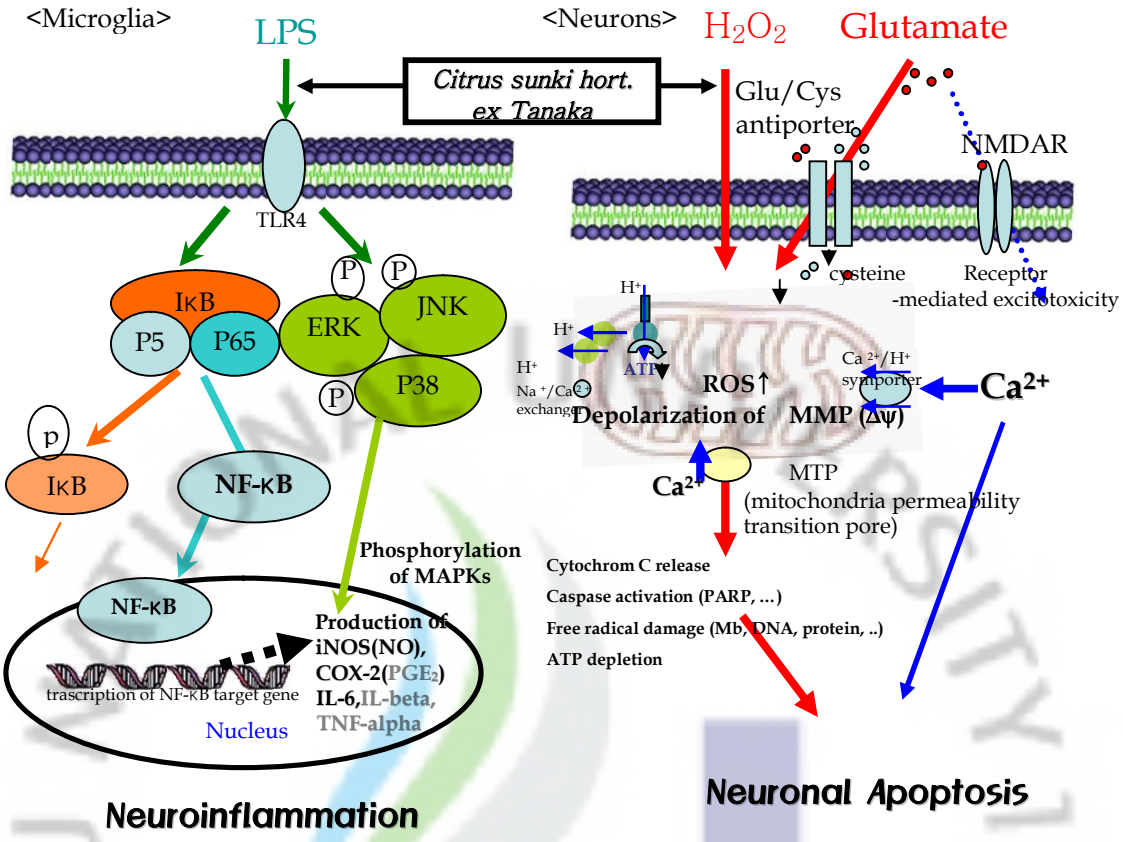


Fig.13. Possible influence of citrus peel extract in LPS-induced neuroinflammation model and oxidative stress model.

2.2. Citrus peel extract inhibited citrus peel extract on oxidative stress-induced cell death in HT22 neurons.

As shown in Fig 10,11 levels of MTT reduction were decreased after treatment with H_2O_2 or glutamate. In addition, cell viability was increased in citrus peel extract treat group in a dose dependent manner. It suggest that citrus peel extract can protect neuronal cell death from oxidative stress.

2.3. Citrus peel extract attenuated H_2O_2-induced alteration of caspase-3 and PARP in HT22 neurons.

Finally, we also examined the expression of procaspase-3 and PARP by Western blot analysis since it is known as the major effecter of the apoptotic process. The cells were treated with H_2O_2 for 12 h after citrus peel extract pretreatment. H_2O_2 decreased the expression of procaspase-3 and PARP. This effect was rescued by citrus peel extract (Fig 12).

Ⅳ. DISCUSSION

Citrus fruit peel has been used in traditional Asian medicine for centuries. Recently, the anti-inflammatory activities among 20 citrus fruit peel extracts are significantly correlated with the content of nobiletin (Choi et al., 2007). Nobiletin is a citrus polymethoxylated falvonoid extracted from citrus, and has several reported biological effect including cognition and memory (Matsuzaki et al., 2006; Nakajima et al., 2007).

A major focus on research neurodegenerative disease is to investigate the inflammatory processes. Inflammatory processes are associated with the pathophysiology of many neurodegenerative disease, including Alzheimer's disease (AD). It has been studied that abnormalities in the production of inflammatory mediators, such as NO and IL-6, may play roles in many inflammatory lesions. These inflammatory mediators are released in the brain following many different neurophathological stiumuli. Activated microglia contribute secondarily to inflammation-mediated tissue destruction via the released of cytokines such as IL-6, TNF-α. Among these mediators, NO and IL-6 have relations with iNOS and COX-2. COX is an enzyme that catalyzes the conversion of arachidonic aicd to prostaglandin H2, a precursor for a variety of biologically active mediators, such as PGE2, prostacyclin and thromboxane A2. COX exists as two major isoenzymes: COX-1, which is a constitutive cyclooxygenase, and COX-2 which is an inducible cyclooxygenase. COX-2 is the predominant cyclooxygenase at sites of inflammation.(Mitchell et al., 1995;Smith et al., 1996), and was produced in macrophages and endothelial cells in response to various stimuli, such as neuronal activity, proinflammatory cytokines.

In present study we examined the biological effects of citrus peel extract on the production of inflammatory mediators in murine macrophage BV2 microglia which was stiumulated with LPS. We found that citrus peel extract significantly inhibited LPS induced increase of NO and IL-6. And it also blcked LPS induced overexpression of iNOS, and COX-2. It was evidenced that MAP kinases play a key role in the regulation of cell growth and

differentiation and in the control of cellular responses to cytokines and stresses, as well as in the activation of NF-κB. Moreover, MAP kinase is known to be important in iNOS and COX-2 expression. To further understand the molecular mechanism of citrus peel extract activity in microglia, we examined the effect of citrus peel extract on the LPS-induced phosphorylation of MAP kinase and IκB in BV2 microglia using Western blot analysis. All of our results indicated that citrus peel extract effectively inhibited LPS-induced activation of BV2 microglia.

In addition, ROS are associated with neuroinflammatory and neurodegenerative processes(Brown and Bal-Price,. 2003; Floyd,. 1999; Floyd, R.A., 2002.). The cells exposed to ROS exhibited distinct features of apoptosis, such as loss of mitochondrial membrane potential ($\Delta\psi$), activation of caspase-3 and PARP cleavage. Physiologically, H_2O_2, a freely diffusible form of ROS, is produced by many intracellular reactions (Cai, H., 2005), and is implicated in both apoptosis and necrosis in various cells (Whittemore et al., 1995; Li et al., 2000; Cole and Perez-Polo., 2002). Glutamate is a major excitatory neurotransmitter in the brain and elevated leavel of extracellular glutamate are neurotoxic. Two pathways for glutamate toxicity have been described, receptor-mediated excitotoxicity and excitotoxicity-independent non-receptormediated oxidative glutamate toxicity . A variety of in vitro models like HT22 neurons, lacking a fully functional excitoxicity pathway, have been used to specifically study oxidative glutamate toxicity-induced cell death. Thus, glutamate induced oxidative stress is more physical than hydrogen peroxide.

In this study we investigated that neuroprotective effects of the citrus peel extract on two different kinds of oxidative stresss , H_2O_2 and glutamate. It was found that citrus peel extract effectively inhibited H_2O_2 and glutamate-induced ROS degeneration and cell death. It also inhibited H_2O_2-induced loss of MMP($\Delta\psi$). Citrus peel extract was found to inhibit the H_2O_2-induced activation of caspase 3 , which is further demondstrated by the cleavage of poly ADP-ribosy polymerase(PARP). These results suggest that citrus peel extract protects

neurons from oxidative stress. Fig 13 represents a schematic rationale of the present discussion about citrus peel extract in microglia and neurons.

All of these results suggest that ethanol extracts of Citrus sunki hort. ex Tanaka peel has neuroprotective acitivity and therapeutic potentials against neuroinflammation and neuronal oxidative stress via regulation of microglial activation and neuronal protection from glutamate-induced oxidative stress.

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Ⅵ. ABSTRACT IN KOREAN

감귤류 과피는 nobiletin, tangeretin 등 항염, 항산화 작용이 있는 플라보노이드 성분을 다량 함유하고 있다. 이 연구에서는 진귤 과피 에탄올 추출물의 항염 작용과 산화적 스트레스에 대한 신경보호작용을 조사하였다.

진귤 과피 에탄올추출물이 LPS로 활성화된 미세 신경교세포(BV2 microglia)에서 염증성 인자로 알려진 NO와 IL-6 발현의 억제효과를 조사하였고 또 이와 관련된 iNOS와 COX-2의 단백질 발현을 조사하였으며 이와 관련된 기전인 NF-κB와 MAPKs의 인산화 억제활성을 측정하여 항염증 기전을 탐색하였다. 연구결과에 의하면 진귤 과피 에탄올 추출물은 BV2 microglia에서 NO의 생성을 효과적으로 억제하였고 IL-6의 mRNA 발현을 억제하였으며 iNOS, COX-2의 protein level에 대해 강한 억제효과를 나타내었다. 이는 또 cytosol에서의 IκB의 degradation을 억제하였으며 MAPKs의 인산화를 억제하였다.

과산화수소(H_2O_2)와 glutamate로 유도된 oxidative stress모델에서 진귤 과피 추출물은 세포 내 활성 산소 종 (ROS)을 감소시키고 oxidative stress에 의하여 유도된 apoptosis를 억제하였다. 또한 DNA복구효소인 PARP의 감소를 억제하고 시스테인 단백분해효소인 caspase 3의 활성화를 억제하는 작용을 나타내었다.

이상의 연구결과를 통하여 진귤 과피 에탄올 추출물은 미세 신경교세포의 활성 억제와 ROS를 감소를 통하여 신경염증과 glutamate에 의한 산화적 손상에 대한 신경보호작용을 나타낸다고 결론 지을 수 있으며, 이는 퇴행성 뇌 질환의 예방, 기능개선 및 치료제 개발의 기초자료로 활용할 수 있을 것으로 본다.

Ⅶ. 감사의 글

대학원에 들어와서부터 이 논문을 작성하기까지 많은 도움과 격려를 주신분들에게 이 글을 빌어 감사의 마음을 전합니다.

우선 석사과정 2년 동안, 항상 힘이 되어준 저의 가족과 친구들과 너무나도 부족한 저를 언제나 믿어주시고 가르쳐 주신 은수용 교수님께 마음 깊이 감사의 말씀을 드립니다. 그리고 연구와 강의로 항상 바쁘신 가운데에도 저의 학위 논문 심사를 맡아주시고 많은 조언을 해주시는 박덕배 교수님과 정성철 교수님께 진심으로 감사 드립니다.

이 논문의 수행과 완성이 있기까지 애정과 관심을 보여주신 강희경 교수님, 유은숙 교수님, 고영상 교수님, 조문제 교수님, 현진원 교수님, 조익현 교수님, 이영기 교수님...모든 교수님들에게 진심으로 감사 드립니다. 그리고 박미경, 강경아, 장예, 김영미, 임희경, 구정은, 현재희, 이혜자, 최지강, 최수길, 김상철, 강경진, 강정일 등 대학원 선배들에게 고마운 마음을 전합니다. 나의 동갑내기 김진영, 김민경, 김보연, 최연희와 우리 실험실 새 맴버인 윤실에게도 고맙다는 말을 전하며 유일한 동기인 지홍이와 홍나영, 부혜진, 고동옥 등 대학원생들에게도 감사의 마음을 전합니다.

2년동안 대학원 생활을 하면서 때로는 힘들고 외로워 포기하고 싶을때도 많았지만 그럴 때마다 저에게 힘을 준 많은 분들 덕에 잘 이겨낼 수 있었던 것 같습니다. 모두들 언제나 건강하시고 행복하세요...

감사합니다.