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Protective Effect of α-Mangostin on High Glucose Induced Endothelial Cell Apoptosis

Kanjana JITTIPORN[1,*], Primchanien MOONGKARNDI[2], Jutima SAMER[3] and Wisuda SUVITAYAVAT[3]

[1]Department of Cardio-Thoracic Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand
[2]Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[3]Department of Physiology, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

([*]Corresponding author's e-mail: kanjanaj@nu.ac.th)

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Abstract

α-mangostin is a phenolic compound from pericarp of mangosteen. It has prominent anti-oxidant properties. Oxidative stress has been shown to be a major factor that disrupts cell functions including endothelium. High glucose (HG) induced ROS production plays a key role in endothelial cell apoptosis. However, the effect of α-mangostin on HG induced apoptosis has not been studied yet. This study demonstrates the effect of α-mangostin in HG induced human umbilical vein endothelial cells (HUVECs) apoptosis. The non-toxic dose of α-mangostin was determined using a MTT assay. Intracellular reactive oxygen species (ROS) and cell apoptosis were evaluated using DCF-DA and TUNEL assays, respectively. The signaling of α-mangostin was elucidated by western blotting. α-mangostin significantly and, dose-dependently, decreased HG induced ROS formation. Also, α-mangostin significantly attenuated HG induced endothelial cell apoptosis. In addition, α-mangostin suppressed HG induced apoptosis via JNK and p38-MAPK. According to our results, α-mangostin attenuated HG induced endothelial cell apoptosis through inhibition of phosphorylation of JNK and p38-MAPK.

Keywords: α-mangostin, high glucose, oxidative stress, apoptosis

Introduction

Microvascular and macrovascular vascular disease is a major complication of diabetes mellitus. High glucose affects cell growth and cell death. Numerous studies have claimed HG enhances reactive oxygen species (ROS) production resulting in endothelial cell apoptosis. Endothelial apoptosis is a cellular event leading to endothelial dysfunction. It is an initial step in several pathologies such as atherosclerosis, hypertension and coronary artery disease [1]. Hence, ROS has a pivotal role in vascular endothelial cell apoptosis. The attenuation of ROS formation is a plausible therapeutic strategy.

Mangosteen (*Garcinia mangostana* Linn) is a tropical fruit that is widespread in Southeast Asia including Indonesia, Malaysia, India and Thailand. Numerous studies have demonstrated that xanthone, a component that exists in the pericarp of mangosteen, has anti-tumor [2-8], anti-inflammation, anti-allergy [3], and anti-oxidant activity [3,9-10]. α-mangostin, the major bioactive component, has been shown to attenuate oxidative stress and myocardial damage in myocardial ischemic/reperfusion injury [11] and isoproterenol induced myocardial infarction in rats [12]. Our previous study showed that α-mangostin decreased hypoxia induced ROS formation in bovine retinal endothelial cells resulting in inhibition of retinal neovascularization [13]. Recently, α-mangostin was shown to attenuate oxidative stress in H_2O_2 stressed retinal pigment epithelial cells and in the retina of light-damaged mice [14].

Mitogen activated protein kinase (MAPK) signaling is important for the maintenance of the cells. c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase, subfamily of MAPK, are involved in various processes including cell proliferation, survival and apoptosis. ERK principally regulates cell survival, whereas JNK and p38 are mainly involved in apoptosis [15]. However, the protective effect of α-mangostin on HG induced endothelial cell apoptosis has not been studied. Thus, the objective of this study was to examine the effect of α-mangostin in HG induced HUVECs apoptosis.

Materials and methods

Chemicals

M199 medium, low serum growth supplement (LSGS), fetal bovine serum (FBS), 0.05 % trypsin, MTT, penicillin and streptomycin were purchased from Gibco-Invitrogen (Grand Island, NY, USA). 2', 7' dichlorodihydrofluorescein diacetate (DCF-DA) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and apoptosis assay kit was purchased from Merck (Mendota Heights, MN, USA).

Plant extraction

The isolation of α-mangostin was described previously [16]. Briefly, mangosteen powder (1 kg) was macerated with ethanol (EtOH) at 60 °C. The crude extract was then filtered and concentrated by a vacuum rotary evaporator at 45 °C. Then, EtOH extract was partitioned with ethyl acetate (EtOAc). The EtOAc extract was chromatographed on a silica gel column chromatography and eluted with gradient solvent system by gradually increasing the polarity. The fractions composed of α-mangostin, detected by thin layer chromatography (TLC), were pooled and rechromatographed with the same solvent system. The physicochemical properties of α-mangostin were determined and confirmed by the melting point, UV spectroscopy, and ^{1}H-NMR ^{13}C-NMR, DEPT 135-NMR, IR and MS spectra. A 98 % pure α-mangostin was dissolved in DMSO. The final concentration of DMSO was less than 0.006 %.

Cell culture

HUVECs were purchased from Thermo Fisher Scientific Company. Cells were cultured in a M199 medium supplemented with 10 % FBS, 5 % LSGS, 100 U/ml penicillin and 100 µg/ml streptomycin in 5 % CO_2 at 37 °C. All experiments were performed in triplicate.

Toxicity of α-mangostin assay

About 2×10^4 cells/ml HUVECs were seeded in 96 well plate in M199 medium supplemented with 10 % FBS, 5 % LSGS, 100 U/ml penicillin and 100 µg/ml streptomycin (complete medium) for 24 h, and then replaced the medium with M199 medium supplemented with 5 % LSGS, 100 U/ml penicillin and 100 µg/ml streptomycin (serum free medium) overnight. The cells were incubated with or without α-mangostin in the same medium at doses 10, 100, 1000 and 2000 nM for 72 h. At the end, the cells were treated with 5 mg/ml MTT at 37 °C in 5 % CO_2 for 4 h. After that, the medium was replaced with 50 µl DMSO. Optical density was determined at 540 nm by using a microplate reader.

2', 7' dichlorodihydrofluorescein diacetate (DCF-DA) assay

To determine the effect of α-mangostin in suppressing HG induced ROS formation, HUVECs were seeded on a 24 well plate in complete medium for 24 h. After serum starvation, cells were incubated with or without α-mangostin at dose 10 and 100 nM for 30 min and then washed with PBS. Subsequently, cells were incubated with 20 µM DCF in a serum free medium for 30 min and then washed with PBS. 25 mM glucose medium (high glucose medium; HG) or 5 mM glucose medium (normal glucose medium; NG) was added and incubated for 10 min. The 5 images were taken at 200× magnification using a fluorescence microscope. The fluorescence intensity was determined by Image J software.

TUNEL assay

Cells were seeded on a gelatin coated 18 mm coverslip in a 12 well plate in a complete medium. At 70 % confluence, the medium was replaced with a serum free medium overnight. After starvation, cells were pretreated with or without α-mangostin and in NG or HG medium for 72 h. Apoptotic cells were verified using the TUNEL assay according to the manufacture's recommendation. Five area fluorescent images were taken at 400× magnification. The number of TUNEL positive cells was counted manually.

Western blot

As described previously [13], briefly, cells were extracted with RIPA buffer. 20 µg proteins were separated and transferred onto polyvinylidine difluoride (PVDF) membranes. Subsequently, the membranes were incubated in primary antibodies against phospho-JNK, total JNK, phospho-p38 and total-p38 at 4 °C overnight. Horseradish peroxidase-conjugated secondary antibodies against primary antibodies were added. The protein bands were verified using chemiluminescence.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was determined by using ANOVA. Post-hoc analysis of individual group differences was performed using a Tukey test and the level of statistical significance was *p*-value less than 0.05.

Results and discussion

Cytotoxicity

Firstly, the non-toxic dose of α-mangostin was determined for treatment of HUVECs by MTT assay. α-mangostin concentrations at 1000 and 2000 nM reduced cell survival significantly. However, cell viability was not significantly changed by 72 h treatment with α-mangostin at concentrations of 10 and 100 nM (**Figure 1**). Hence, α-mangostin concentrations of 10 and 100 nM were used for further experiments.

α-mangostin suppresses oxidative stress

ROS formation plays a pivotal role in cell death. Next, the effects of α-mangostin on HG induced ROS formation was determined using a DCF-DA assay. α-mangostin concentrations at 10 and 100 nM significantly suppressed HG induced ROS production in a dose-dependent manner (**Figures 2A** and **2B**).

α-mangostin attenuates endothelial cell apoptosis

HG induced endothelial cell apoptosis leads to endothelial dysfunction. The effect of α-mangostin on HG induced endothelial cell apoptosis was verified using a TUNEL assay. α-mangostin at a dose of 100 nM significantly inhibited HG induced endothelial cell apoptosis (**Figures 3A** and **3B**).

α-mangostin attenuates HG induced apoptosis through JNK-MAPK and p38-MAPK signaling

Mitogen-activated protein kinase (MAPK) is the major downstream signaling mechanism of HG induced apoptosis especially, JNK-MAPK and p38-MAPK. The signaling pathway of α-mangostin on HG induced apoptosis in HUVECs was determined and shown that 100 nM α-mangostin significantly inhibited phosphorylation of JNK and p38-MAPK (**Figures 4A** and **4B**).

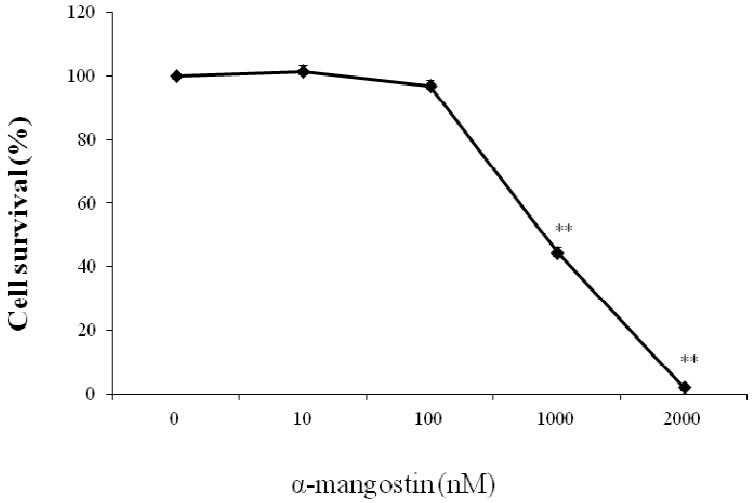
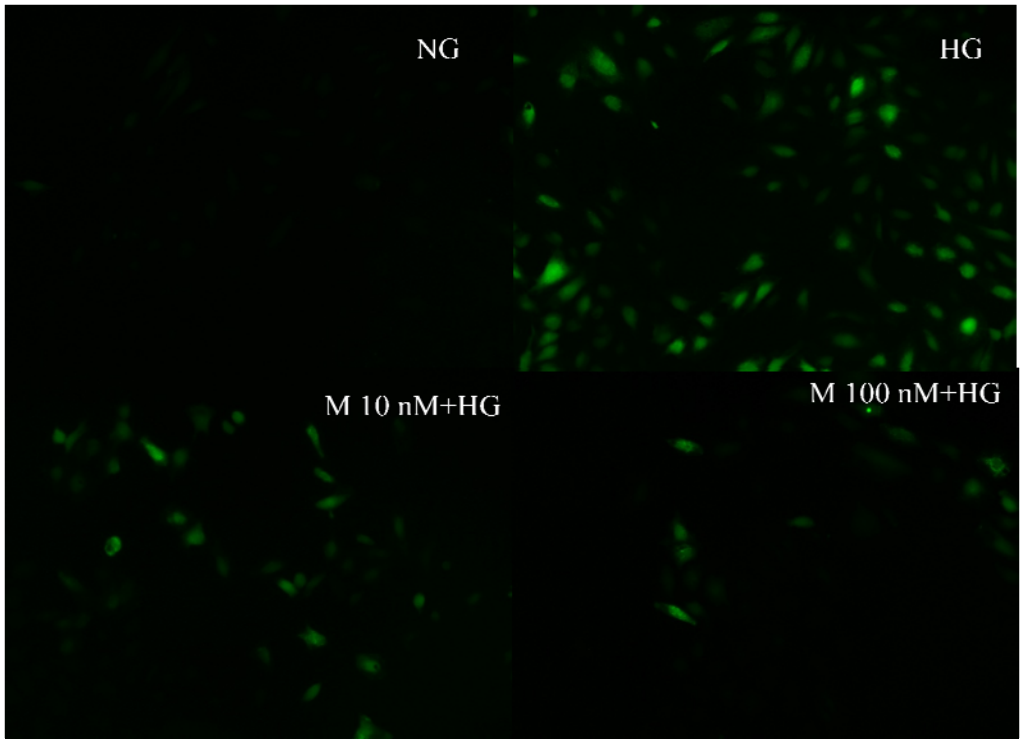


Figure 1 Impact of α-mangostin on HUVECs viability. HUVECs were treated with various concentrations of α-mangostin for 72 h. Cell viability was assessed by MTT assay (n = 3 $^{**}p < 0.01$ vs control).

(A)



(B)

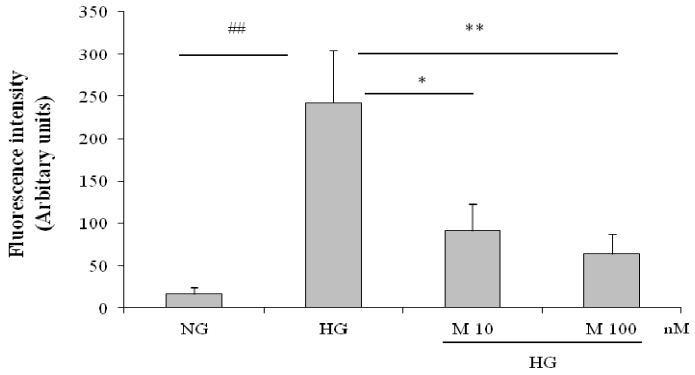
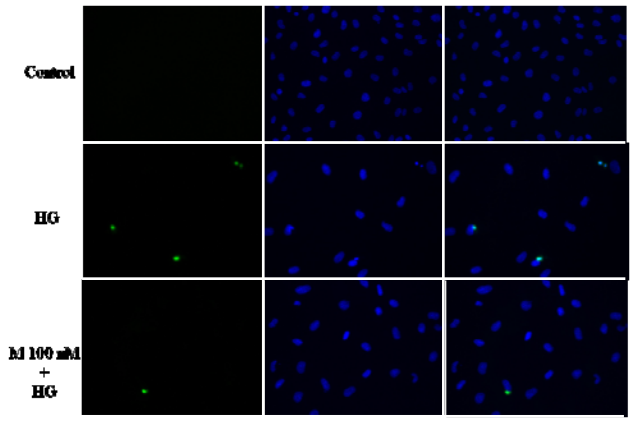


Figure 2 Effect of α-mangostin on HG induced ROS production. HUVECs were incubated with normal glucose (NG) or high glucose (HG) medium in the presence or absence of α-mangostin. Intracellular ROS was determined using DCF-DA. (A) Fluorescence microscope images of DCF-DA staining. (B) Quantification of fluorescence intensity was determined by Image J software (n = 3 $^{\#\#}p < 0.01$ vs control, $^{*}p < 0.05$, $^{**}p < 0.01$ vs HG).

(A)



(B)

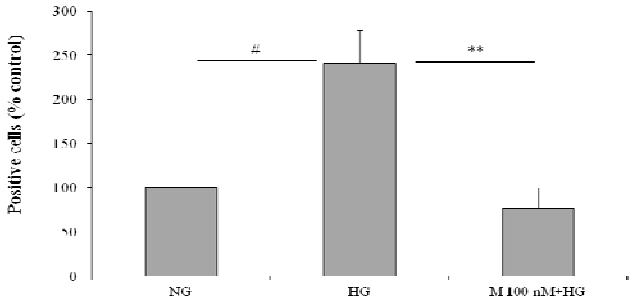


Figure 3 Effect of α-mangostin on HG induced apoptosis using a TUNEL assay. HUVECs were incubated with normal glucose (NG) or high glucose (HG) medium for 72 h in the presence or absence of α-mangostin. (A) Fluorescence microscope images of positive cells. (B) Quantification of positive cells was determined manually (n = 3 $^{\#}p < 0.05$ vs control, $^{**}p < 0.01$ vs HG).

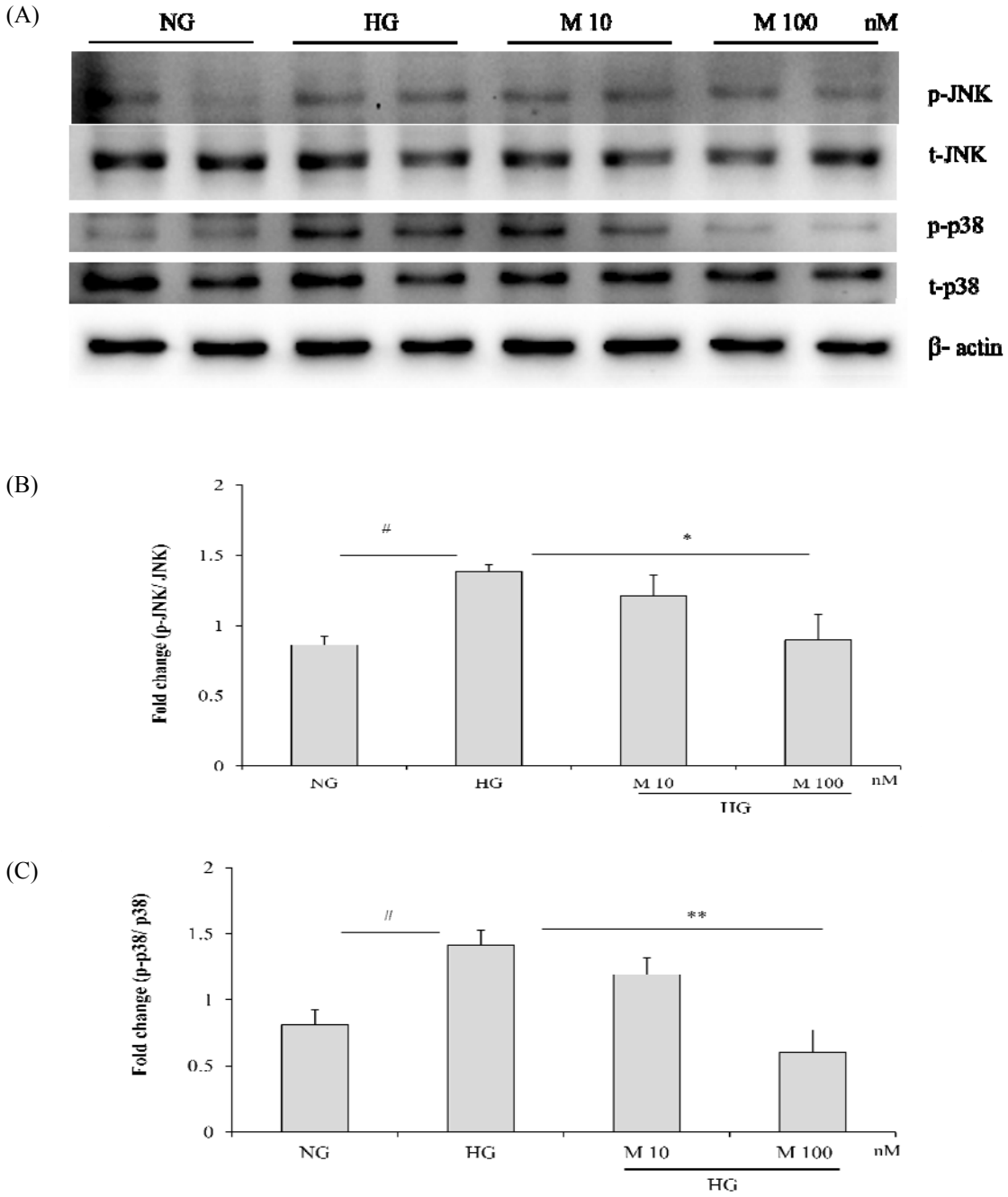


Figure 4 Effect of α-mangostin on HG induced apoptosis signaling. HUVECs were treated with normal glucose (NG) or high glucose (HG) medium for 15 min in the presence or absence of α-mangostin. Cell lysates were subjected to SDS-PAGE for phosphorylation of JNK and p38 (A). Densitometric quantification of JNK (B) and p38 (C) was determined by Image J software. (n = 3 $^{\#}p < 0.05$ vs control, $*p < 0.05$, $**p < 0.01$ vs HG).

Discussion

This study has demonstrated that α-mangostin inhibited HG induced endothelial apoptosis via reduction of oxidative stress. The inhibitory effect of α-mangostin is mediated through attenuating the activation of phosphorylation of JNK and p38-MAPK.

It has been found that HG increased ROS production from the mitochondria [17] and NADPH oxidase [18]. Excessive ROS generation caused endothelial cell apoptosis leading to endothelial cell dysfunction. It has been shown that α-mangostin, polyphenolic xanthone isolated from mangosteen pericarp, has an anti-oxidant action through radical scavenging [3]. Our previous study showed that α-mangostin suppressed hypoxia induced retinal oxidative stress resulting in attenuation of retinal neovascularization [13]. Numerous studies have demonstrated that α-mangostin suppresses ROS production leading to a renoprotective effect in cisplatin induced nephrotoxicity [19] and cardioprotective effects in models of cardiac reperfusion injury [11] and in isoproterenol induced myocardial infarction in rats [12]. In addition, α-mangostin decreased peroxidation in rat brain tissue [20]. Recently, it has been shown that α-mangostin has a retinoprotective effect in light damaged mice models [14]. Similarly, this study showed that α-mangostin inhibits HG induced ROS generation which indicates its anti-oxidant activity.

Numerous studies have demonstrated that α-mangostin has pro-apoptotic action to kill cancer cells including human oral squamous cell carcinoma [21], human tongue mucoepidermoid carcinoma [22], human breast cancer T47D cells [23], MCF-7 and MDA-MB-231cells [24], human colon cancer cells [25], human colorectal cancer DLD-1 cells [26] and human leukemia HL60 cells [27]. α-mangostin was shown to attenuate free fatty acid induced steatosis on liver cells through inhibition of caspase 3 and 9 activities [28]. However, the present study, for the first time, demonstrated that α-mangostin has anti-apoptotic action on HG induced endothelial cell apoptosis.

HG induced endothelial cell apoptosis through activation of phosphorylation of JNK [29] and p38-MAPK [30-31] and inhibition of phosphorylation of Akt [32]. In the present study, α-mangostin attenuated HG induced apoptosis as detected by TUNEL assay. α-mangostin reduced HG induced endothelial apoptosis through inhibition of phosphorylation of JNK and p38-MAPK but not ERK1/2-MAPK and Akt (data not shown). Taken together, this study indicated that α-mangostin inhibited HG induced ROS production and endothelial apoptosis via suppression of JNK and p38-MAPK phosphorylation.

Conclusions

α-mangostin inhibited HG induced endothelial apoptosis through inhibition of phosphorylation of JNK and p38-MAPK.

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