Protective Effect of *Phyllanthus Emblica* Fruit Extract Against Hydrogen Peroxide-Induced Endothelial Cell Death

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Numerous antioxidants from natural products have been shown to lower ROS levels and enhance vascular endothelial function. The fruits of Phyllanthus emblica are well-known in possessing antioxidative properties but its role and mechanisms in the protection of vascular endothelial cells from ROS damage have not yet been established. The present study was aimed to determine the possible protective effect of P. emblica fruit extract (PE) on human EA.hy926 endothelial cell death induced by hydrogen peroxide (H_2O_2) and PE protective mechanisms. Following incubation of endothelial cells with 300 microM H_2O_2 for 2 h, cell viability was decreased to 50.65 $\pm$ 0.94% and intracellular ROS levels was increased to 159.01% $\pm$ 6.27% as measured by MTT assay and DCF fluorescent intensity, respectively. Cytotoxic effect of PE was not observed in the range of 0.1 to 100 microM. Pretreatment with PE (20 to 100 microg/mL) for 48 h significantly ameliorated the cytotoxic effect of H_2O_2 and attenuated the excessive intracellular ROS formation in endothelial cells. In addition, western blot analysis revealed that PE pretreatment (40 microg/L) induced Akt phosphorylation but did not activate NF-kappaB pathway. These findings suggest that PE could effectively protect human endothelial cell death induced by H_2O_2 via modification of ROS-related mechanism along with activation of PI3K/Akt pathway. However, the value of this plant in vivo needs further investigations in supporting them to be developed as nutraceuticals for cardiovascular disease prevention.

Keywords: *Phyllanthus emblica, Endothelial cell, Reactive oxygen species, Cell death*

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Cardiovascular diseases (CVDs) are the primary cause of morbidity and mortality in the world. In 2008, it was estimated that 17.3 million people died from CVDs and the incidence has been on the raise to 23.6 million people in 2030[1]. Some risk factors of CVDs initiate endothelial pathophysiology resulting in functional impairment. The normal function of endothelial cells include vascular homeostasis that implicated in the regulation of vascular tone, leukocyte adhesion, platelet activity and thrombosis. Endothelial dysfunction in response to excess reactive oxygen species (ROS) leads to the development of vascular disease especially atherosclerosis[2,3]. Therefore, the assessment of endothelial function is an indicator of risk prediction in cardiovascular complications.

Among all ROS, hydrogen peroxide (H_2O_2) is a relatively stable oxidant that can penetrate through the vascular membrane. Several studies have shown that high concentration of H_2O_2 induces endothelial cell death via enhancement of transferrin receptor-dependent intracellular iron uptake, activation of Janus Kinase (JNK)/c-Jun and Fas pathway and induction of mitochondrial DNA damage[4,5]. Most cells respond to this oxidative insults either by activation of survival signaling such as Akt pathway or increase endogenous antioxidant defense mechanisms. It has been shown that Akt associates with multiple pathways which promote endothelial cell survival from oxidative stress such as vascular endothelial growth factor receptor 2 (VEGF-R2)/PI3K/Akt pathway[6] and Akt/eNOS pathway[7].

Phyllanthus emblica L (syn. *Emblica officinalis* Gaertn), commonly known in Thai as Makham-pom, is a deciduous tree belonging to the

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Euphorbiaceae family. Several parts of the tree including roots, bark, leaves, stem node and fruits are commonly used in traditional medicine[8]. Bioactive constituents consist of ascorbic acid, tannins, flavonoids and alkaloids[9]. In Ayurvedic and Thai folk medicine, fruits of *P. emblica* are used as antitussive, expectorant, antipyretic, refrigerant, laxative, antidiarrhoeic, diuretic, and antiscorbutic agents[10]. Recently, *P. emblica* has been evaluated for its numerous pharmacological effects such as hypochloesterolemic, hypoglycemic, antiulcerative and antioxidative effects[11-13]. However, there is little information of *P. emblica* related to the enhancement of endothelial survival. The present study aimed to evaluate the protective effect of *P. emblica* fruit extract on H_2O_2-induced endothelial cell death and explore the potential signaling pathway of *P. emblica* fruit extract on promoting endothelial cell survival.

Material and Method

Materials

Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin, fetal bovine serum (FBS) and HRP-conjugated goat anti-mouse IgG were purchased from Invitrogen. HRP-conjugated goat anti-rabbit IgG was purchased from Upstate. 3-(4,5-Dimethythiazolyl-2)-2, 5-diphenyltetrazoliumbromide (MTT), aluminium chloride, catechin, 22, 72-dichlorofluorescin diacetate (DCFH-DA), gallic acid, HEPES, EDTA, EGTA, protease inhibitor cocktail, sodium orthovanadate and Igepal were purchased from Sigma. Tween 20 and 1, 4-Dithiothreitol were purchased from Calbiochem. Folin-Ciocalteu's phenol reagent was purchased from Fluka. Hydrogen peroxide and sodium nitrite were purchased from Merck. Rabbit polyclonal NF-kappaB p65 and mouse monoclonal beta-actin, p-Akt (Ser 473) and Akt antibodies were purchased from Santa Cruz Biotechnology. ECL prime western blotting detection reagents was purchased from Amersham Biosciences.

Preparation of P. emblica fruit extract (PE)

Fresh fruits of *P. emblica* were purchased from the market in Bangkok, Thailand. After separating their seeds, the fruit pulps were pressed to make juice using automatic juice extractor. The sample was centrifuged at 3,000 g, 4°C for 10 min and the supernatant was filtered through the Whatman No. 1 filter paper. The filtrate was lyophilized and kept at -80°C in the light protecting package. The lyophilized powder was weighed and calculated for percentage yield. The aqueous stock solutions of PE (10 microg/mL) were prepared freshly before use.

Determination of flavonoid content

Flavonoid content of the fruit extract was determined using the aluminium chloride colorimetric assay, using catechin as a reference standard[14]. The fruit extract or standard solution was placed on 96-well non-sterile microplate at a volume of 100 microL/well and subsequently added 6 microL of 5% $NaNO_2$ to each well. After incubation for 5 min at room temperature, 6 microL of 10% $AlCl_3$ were added and mixed in each well. The solution was kept at room temperature for 1 min, followed by addition of 1 N NaOH at 40 microL/well. The total volume of each well was adjusted to 200 microL with distilled water. Absorbance of these sample reactions was measured at 510 nm using a microplate reader (Synergy HT, BioTek). Flavonoid content calculated from the standard curve was expressed as mg of catechin equivalent (CE) per g of lyophilized powder.

Determination of total phenolic content

Total phenolic content of the fruit extract was determined using the colorimetric Folin-Ciocalteu assay, using gallic acid as a reference standard[15]. The fruit extract or standard solution was added in a test tube at a volume of 20 microL and then mixed with 1.58 mL distilled water. Thereafter, 100 microL of Folin-Ciocalteu's phenol reagent and 300 microL of 20% Na_2CO_3 were respectively added. The mixture was incubated in a water bath setting temperature at 40°C for 30 min. After incubation, 200 microL of the mixture were transferred into 96-well non-sterile microplate and the absorbance at 765 nm was determined using a microplate reader (Synergy HT, BioTek). The total phenolic content obtained from experiment was expressed as mg of gallic acid equivalent (GAE) per g of lyophilized power.

Cell culture

The human EA.hy926 endothelial cell line was obtained from American Type Culture Collection (ATCC), USA. The cells were cultured in DMEM supplemented with 10% FBS, 100 units/mL penicillin and 100 microg/mL streptomycin. Cells were maintained at 37°C in a humidified air containing 5% CO_2. Medium was changed every 2-3 days and cells were subcultured when 80% confluence.

Cell viability assay (MTT assay)

The capability of mitochondrial enzyme is used to determine cell viability under the colorimetric analysis. EA.hy926 cells were trypsinized when 80%

confluence and seeded on 96-well culture plate at a density of 10^4 cells/well with DMEM containing 1% FBS. After overnight incubation, cells were treated with PE at the indicated concentrations for 48 h. The media were then removed and replaced with fresh media (1% FBS) containing 300 microM H_2O_2 and allowed for incubation for 2 h at 37°C. After removal of the incubation media, cells were washed with phosphate buffered saline (PBS) and subsequently added with 0.25 mg/mL MTT in medium containing 1% FBS for 3 h. At the end of the experiment, the media were discarded and replaced with 100 microL DMSO, gentle shaking for 2-3 min until formazan crystals were completely dissolved. Absorbance was measured at 550 nm by using a microplate reader (Synergy HT, BioTek).

Determination of intracellular ROS formation

DCFH-DA was used for intracellular ROS measurement. The cells were plated on 96-well culture plate at a density of 10^4 cells/well in DMEM with 1% FBS and incubated overnight at 37°C. Cells were then pretreated with PE at various concentrations for 48 h and exposed to 300 microM H_2O_2 for 2 h. Thereafter, cells were washed with PBS and incubated with 50 microM DCFH-DA solution for 30 min in dark. After removal of the solution, cells were washed three times with PBS followed by addition of 100 microL PBS. Fluorescence was measured at excitation/emission wave lengths of 485/528 nm using a fluorescent microplate reader (Synergy HT, BioTek).

Preparation of cytoplasmic and nuclear extracts

After treatment, cells were washed with PBS and gently scraped into ice-cold PBS. They were spinned down at 10,000 rpm, 4°C for 1 min. The supernatant was discarded and replaced with hypotonic buffer (10 mM HEPES, pH 7.9, 0.1 mM EDTA, 0.1 mM EGTA, 10 mM KCl, 1 mM DTT, 1 mM Na_3VO_4, 1% protease inhibitor cocktail, 0.5% Igepal) for 15 min on ice. Following centrifugation at 12,000 rpm, 4°C for 15 min, the supernatant containing cytoplasmic fraction was collected and stored at -80°C for measurement of p-Akt and Akt level. The pellets were subsequently resuspended in hypertonic buffer (20 mM HEPES, pH 7.9, 1 mM EDTA, 1 mM EGTA, 0.4 M NaCl, 1 mM DTT, 1% protease inhibitor cocktail) for 30 min on ice with agitation from time to time. After cell lysis, the samples containing nuclear fraction were centrifuged at 12,000 rpm, 4°C for 30 min[16]. The supernatant was harvested and stored at -80°C for measurement of NF-kappaB content. The amount of proteins was determined by Bio-Rad protein assay.

Western blot analysis of p-Akt, Akt and NF-kappaB

To evaluate transducer proteins involved in the survival signaling phosphyrylation of Akt and translocation of NF-kappaB was evaluated by western blot analysis. Endothelial cells were treated with PE for 48 h and then exposed to H_2O_2 for 2 h as described above. Cells the equal amounts of protein samples (40 microg of cytoplasmic and 30 microg of nuclear proteins) were separated by 10% SDS-PAGE. They were then transferred onto PVDF membrane using Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell (BioRad, USA) for 1 h at 20 V. The membranes were blocked by solution containing 5% non-fat dry milk in PBST (PBS with 0.1% Tween 20) for 1 h at room temperature and probed overnight at 4°C with the indicated primary antibodies including p-Akt (1:500), Akt (1:2,000), NF-kappaB p65 (1:1,000) and beta-actin (1:2,000) in the blocking solution. Further, the membranes were washed three times with PBST and probed with either HRP-conjugated goat anti-mouse IgG (1:2,000) or goat anti-rabbit IgG (1:1,000) secondary antibodies in the blocking solution for 1 or 1.5 h, respectively. The blots were washed three times with PBST and followed by chemiluminescent detection (Amersham Biosciences). The signals were measured using GeneSnap program in a GeneGnome equipment (Syngene) and quantified by ImageJ software.

Statistical analysis

The results were presented as the mean $\pm$ SEM. GraphPad Prism version 5.01 software was used to analyze the data. All data groups were compared with one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The statistically significant level was considered at $p\text{-value} < 0.05$.

Results

Percentage yield, flavonoid and total phenolic contents of PE

According to the extraction manner described previously, the lyophilized powder product had a yellowish brown color and a percentage yield of 16.1% w/v. This result indicated that 1 g of the extract is equal to 11.98 g of fresh fruit pulp. The contents of flavonoid and total phenol were 32.84 $\pm$ 3.14 mg CE and 323.10 $\pm$ 22.86 mg GAE per g of lyophilized powder, respectively.

Endothelial cell death induced by H_2O_2

To test the oxidative stress condition,

EA.hy926 cells were exposed to various concentrations of H_2O_2 (100 microM to 10 mM) for 2 h and proportion of cell survival was analyzed using MTT assay. Hydrogen peroxide reduced cell viability at concen-0trations 200 microM and above but at concentrations 300 to 1,000 microM, cell viability was reduced at the same extent (data not shown). At 300 microM H_2O_2, cell viability was reduced to 50.65 $\pm$ 0.94% compared with the vehicle treated group. Therefore, this concentration was chosen to induce cell death in the next experiments.

Effect of PE on H_2O_2-induced reduction in endothelial cell viability

EA.hy926 cells were treated with different concentrations of PE (0.1 to 100 microg/mL) for 48 h under normal conditions. The fruit extract did not affect cell proliferation (Fig. 1A). To assess the effect of PE on H_2O_2-treated cells, cells were pretreated with PE for 48 h followed by incubation with 300 microM of H_2O_2 for 2 h and cell survival was determined by evaluation of mitochondrial reductase activity. As shown in Fig. 1B, pretreatment with PE at the lowest concentration effective in cytoprotection (20 microg/mL) significantly increased cell viability in the H_2O_2-treated group. The cellular morphological changes were simultaneously observed under the phase contrast inverted microscope (Olympus DP20). As illustrated in Fig. 2, the H_2O_2-exposed cells were shrinking while PE (20 microg/mL) pretreated cells exhibited a decrease in cell shrinkage when exposed to H_2O_2. Similarly, pretreatment with PE at 40 microg/mL showed improvement in morphological change after H_2O_2 exposure leaning toward the morphology of vehicle control cells.

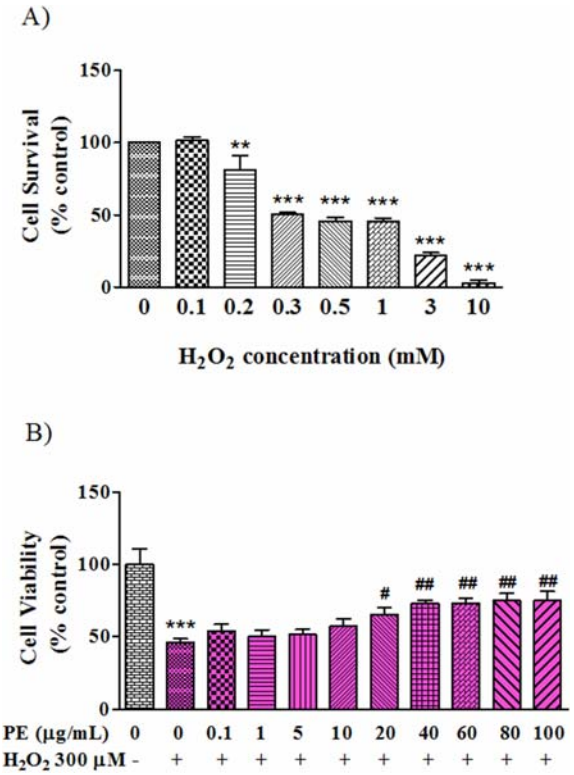

Fig. 1 Effect of PE on EA.hy926 cell viability. A) toxic effect of hydrogen peroxide (H_2O_2) at various concentrations. B) cytoprotective effect of PE at concentration between 1-100 microg/mL. Cell survival was evaluated by MTT assay as described in Materials and Methods. + indicates in the presence of H_2O_2; - indicates without H_2O_2. Data are expressed as mean $\pm$ SEM (n = 3). ***$p < 0.001$ compared to the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the H_2O_2-treated group

Effect of PE on intracellular ROS generation

The intracellular ROS levels in EA.hy926 cells were detected by DCF fluorescence analysis. PE at 0.1 to 20 microg/mL did not influence the fluorescent intensity in a normal condition but at the higher concentrations (40 to 100 microg/mL), a significant lowering in ROS levels was observed (Fig. 3A). When cells were exposed to 300 microM H_2O_2 for 2 h, a marked increase in fluorescent intensity was obtained (159.01 $\pm$ 6.27%). Pretreatment with PE at 40 to 100 microg/mL for 48 h significantly attenuated the H_2O_2-induced ROS generation as illustrated in Fig. 3B.

Effect of PE on p-Akt, Akt and NF-kappaB expression

To test the molecular mechanism of PE extract as cytoprotective compounds against oxidative stress, phosphorylation level of Akt (p-Akt) at serine 473, expression of Akt and the presence of nuclear NF-kappaB p65 were evaluated by western blot analysis. The housekeeping protein beta-actin was used to normalized protein loadings. Fig. 4A shows that treatments with PE alone, H_2O_2 alone or the combination did not induce substantial alterations in Akt expression. Treatment with PE at 40 microg/mL for 48 h prior to 2 h H_2O_2 exposure (300 microM) significantly increased the p-Akt/Akt band intensity ratio (2.20 $\pm$ 0.4) compared with the control (1.00 $\pm$ 0.12) and H_2O_2-treated (1.27 $\pm$ 0.12) groups (Fig. 4B). This indicated that phosphorylation of Akt at serine 473 residue is potentially a mode of action for PE to protect cells against oxidative stress. Although statistically insignificant, it is noteworthy to stress that cells treated with PE (40 microg/mL) solely

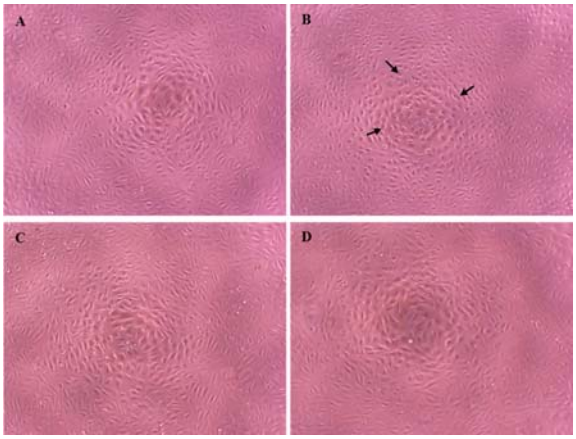

Fig. 2 Morphological change of EA.hy926 cells under the phase contrast inverted microscope with 10X magnification. (A), untreated cells; (B), H_2O_2-exposed cells.; (C), PE 20 microg/mL treated cells; (D), PE 20 microg/mL-pretreated cells exposed to H_2O_2. The arrows indicate cell shrinkage

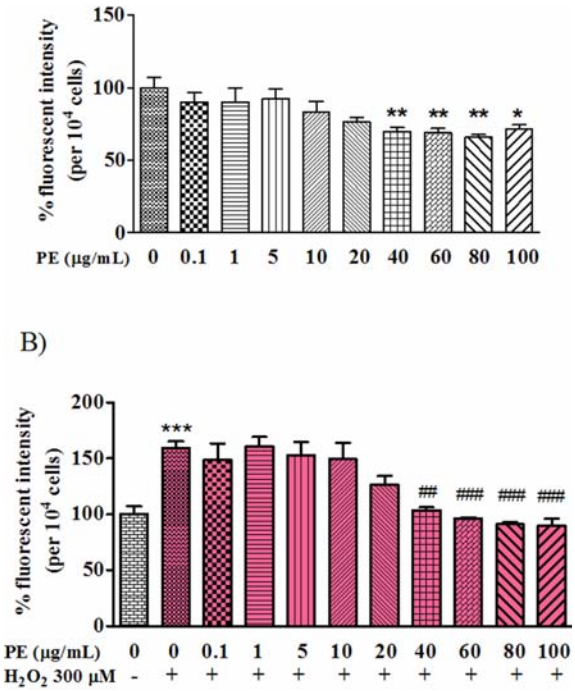

Fig. 3 Effect of PE on intracellular ROS formation under normal (A) and oxidative (B) conditions using DCF fluorescence analysis. EA.hy926 were pretreated with PE at various concentrations for 48 h and then exposed to 300 microM H_2O_2 for 2 h. Detection of ROS level using DCFH-DA was performed as described in Material and Method. + indicates in the presence of H_2O_2; - indicates without H_2O_2. Data are expressed as mean $\pm$ SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared to the control group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to the H_2O_2-treated group

for 48 h seem to increase the Akt signaling (p-Akt/Akt ratio, 1.74 $\pm$ 0.25) but cells treated with H_2O_2 did not show any change in the phosphorylated stage. For the evaluation of signaling through NF-kappaB, PE at both concentrations of 20 and 40 microg/mL exhibited no statistically significant alterations in NF-kappaB p65/beta-actin band intensity ratio (Fig. 5). Similarly, the difference of NF-kappaB p65 expression between H_2O_2-exposed cells and untreated cells did not attain the statistical significance.

Discussion

Oxidative stress is a major cause of cellular damages in many disorders especially in CVDs. In addition to therapeutic interventions, the herbal products with antioxidative property have been shown to be capable of protecting endothelial cell from oxidative damages[17]. The interesting natural product, *P. emblica*, has previously been reported to be effective against doxorubicin toxicity in H9c2 cardiac cells[18] and against ischemic-reperfusion injury in Wistar rats[19]. The present study is the first to exhibit protective effect of this plant extract on an in vitro model of human endothelial cell death induced by H_2O_2. The authors found that endothelial cells incubated with H_2O_2 at the concentration of 300 microM for 2 h reduced cell viability and elevated intracellular ROS production. The morphological appearance of cell shrinkage suggested the early stage related to cell death. Moreover, it appears that PE exerted its cytoprotective role through PI3K/Akt survival signal without changes in NF-kappaB nuclear accumulation. Thus, PE may be useful in the prevention of cardiovascular complications associated with oxidative stress associated with excessive H_2O_2 generation.

Interestingly, the treatment of endothelial cells with PE alone at concentration 40 microg/mL for 48 h markedly suppressed intracellular ROS generation while cell viability was not affected. The 48-h pretreatment with PE before H_2O_2 incubation significantly increased cell viability and inhibited the raised intracellular ROS production. These outcomes suggested that antioxidant activity or activation of endogenous oxidative defense mechanisms is involved in the underlying mechanisms of cytoprotective action of the fruit extract.

These effects were potentially a result of the

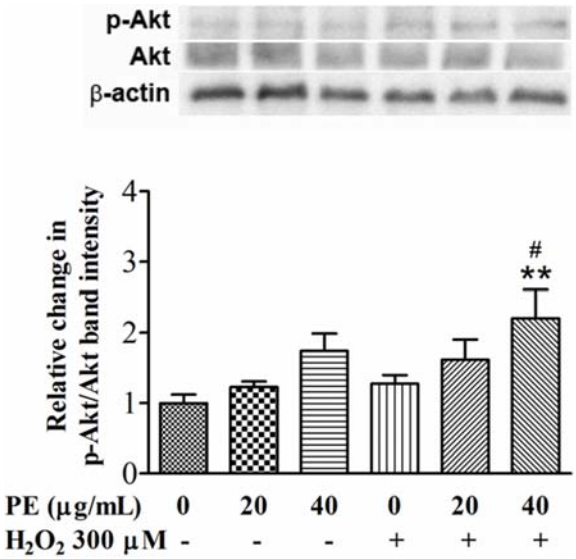

Fig. 4 Effect of PE on p-Akt and Akt expression in EA.hy926. Western blot analysis of p-Akt and Akt is presented as p-Akt/Akt band intensity ratios. EA.hy926 were pretreated with PE at 0-40 microg/mL for 48 h and then exposed to 300 microM H_2O_2 for 2 h. Evaluation of phosphorylated Akt was performed as described in Material and Method. + indicates in the presence of H_2O_2;- indicates without H_2O_2. Data are expressed as mean ± SEM (n = 7). **$p < 0.01$ compared to the control group; #$p < 0.05$ compared to the H_2O_2-exposed group

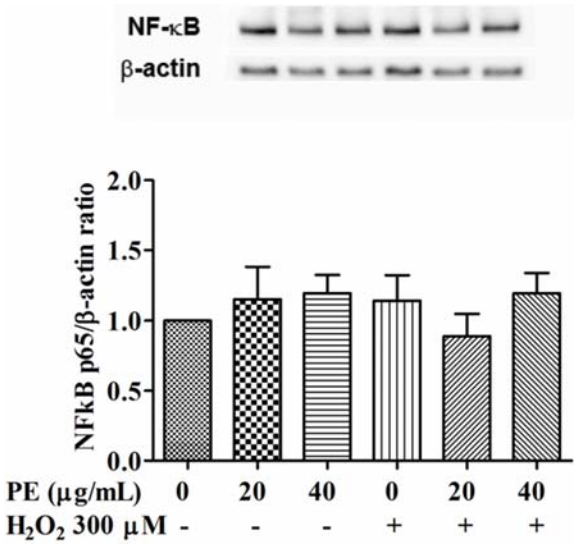

Fig. 5 Effect of PE on NF-kappaB p65 subunit expression in EA.hy926 cells (n = 6) + indicates in the presence of H_2O_2; - indicates without H_2O_2

high content of antioxidants including flavonoid, phenolic compounds, and ascorbic acid in the fruit extract. Certain bioactive constituents of PE have been shown to protect cellular oxidative damage in several models of studies. For instance, corilagin inhibited lipid oxidation[20] and the tannoid emblicanin ameliorated ischemia-reperfusion-induced oxidative stress in rat heart[21]. In addition, it has been shown that ascorbic acid (60 microM) promoted human endothelial cell growth via the ERK-signaling pathway[22]. These constituents of the extract might therefore provide a possible synergistic antioxidative effect.

For the condition used in the present study, it is believed that serum starvation in a period of treatment is helpful for the interpretation of the effects of the extract since FBS contains multiple growth factors which are required for cell growth, proliferation and survival. Hence, cells were maintained in DMEM containing 1% FBS during treatment with the extract and exposure to H_2O_2. Nonetheless, the lower concentration of H_2O_2 used in the present study might be an appropriate dose for inducing endothelial cell death (approximately 25%) and possibly exhibit a significant cytoprotective effect of PE. Since at 300 microM of H_2O_2 seemed to cause excess endothelial cell death (around 50%), the capability of PE on recovery might be masked.

The regulation of cell survival and cell death under physiological and pathological conditions is known to be associated with many signaling proteins such as Akt and NF-kappaB. In the present study, H_2O_2 at 300 microM did not alter both Akt and p-Akt (Ser 473) levels in endothelial cells after 2-h exposure time, suggesting that the signaling pathway by which H_2O_2 at this concentration induced endothelial cell death was not through the reduction of the Akt phosphorylation-mediated survival signaling. Nevertheless, it might be possible that this ROS worked through death signaling pathways. Regarding to the effects of PE, the Akt level was not altered but the phosphorylation of Akt on serine 473 residue was found in PE-pretreated cells under acute oxidative stress. These findings agree with many studies demonstrating that Akt phosphorylation is a pivotal action of antioxidant substances on cellular protection[23]. Hence, the molecular mechanism of cytoprotective function of this fruit extract seemed to be the result of the partial activation the PI3K/Akt signaling pathway through phosphorylation of Akt at serine 473 while the Akt level was not affected.

Regarding PI3K/Akt signaling pathway, one of all downstream signaling molecules of PI3K/Akt

pathway is NF-kappaB transcription factor. Accumulating evidence has shown the connection between PI3K/Akt and NF-kappaB pathways in multiple cells especially in case of inflammatory cytokine induction[24]. However, the correlation between Akt signaling and NF-kappaB under oxidative condition remains controversial. Focusing on endothelial cell culture models studied in this research, the authors found that exposure of EA.hy926 to H_2O_2 300 microM for 2 h did not alter the level of NF-kappaB p65 subunit in nuclei. This is similar to the result of Lakshminarayanan et al (1998) demonstrating that incubation with H_2O_2 at concentrations of 100 and 800 microM for 1 h did not stimulate NF-kappaB p65 nuclear translocation in both HMEC-1 and lung epithelial A549 cells, respectively[25]. In addition, Jaspers et al (2001) reported no significant nuclear translocation of NF-kappaB p65 subunit following induction of bronchial epithelial cells with H_2O_2 (500 microM, 30 min) despite the enhanced activation of IKK complex[26]. As for the molecular cytoprotective mechanism of PE studied, no alteration of NF-kappaB p65 nuclear translocation was observed in response to oxidative stress. This result suggested that the cellular protection of PE against oxidative stress does not influence NF-kappaB activation. Nonetheless, since the investigation of pathways involved in cell survival and cell death was not comprehensive, it is possible that other molecular mechanisms may involve in PE cytoprotection such as p38 MAPK, TRAIL, and mitochondrial stress. Thus, other signaling pathways, particularly the death signals, involving in the effect of H_2O_2 should further be investigated.

Conclusion

The fruit extract of *P. emblica* could effectively promote human endothelial cell survival against H_2O_2 insult through modification of endogenous oxidative defense mechanisms together with activation of Akt phosphorylation in the survival signaling pathway. These actions are possibly attributed by its high antioxidant constituents, including ascorbic acid, flavonoids and phenolic compounds. The beneficial effect of the extract showed in the present study indicates that it could potentially be developed to neutraceuticals for the purpose of preventing cardiovascular disease. However, the value of this plant extract needs further study to warrant these findings.

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Potential conflicts of interest

None.

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ฤทธิ์ของสารสกัดผลมะขามป้อมในการปกป้องการตายของเซลล์เยื่อบุหลอดเลือดจากการเหนี่ยวนำด้วยไฮโดรเจนเปอร์ออกไซด์

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ปัจจุบันพบว่าสารต้านอนุมูลอิสระจากผลิตภัณฑ์ธรรมชาติจำนวนมากสามารถลดระดับ ROS และกระตุ้นการทำงานของเซลล์เยื่อบุหลอดเลือดได้ มะขามป้อมเป็นสมุนไพรชนิดหนึ่งที่มีฤทธิ์ต้านอนุมูลอิสระ แต่ยังไม่มีการศึกษาถึงบทบาทและกลไกในการช่วยปกป้องเซลล์เยื่อบุหลอดเลือดจากสารในกลุ่ม ROS ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อตรวจหาฤทธิ์ของสารสกัดผลมะขามป้อมที่อาจช่วยปกป้องการตายของเซลล์เยื่อบุหลอดเลือดจากการเหนี่ยวนำด้วยสารในกลุ่ม ROS รวมถึงกลไกที่เกี่ยวข้องโดยใช้ไฮโดรเจนเปอร์ออกไซด์เป็นตัวแทนของสารดังกล่าว หลังจากบ่มเซลล์ด้วยไฮโดรเจนเปอร์ออกไซด์ที่ความเข้มข้น 300 ไมโครโมลาร์ เป็นเวลา 2 ชั่วโมง พบว่าเซลล์มีการอยู่รอดลดลงเหลือ 50.65% $\pm$ 0.94% และมีระดับ ROS ในเซลล์เพิ่มขึ้นเป็น 159.01% $\pm$ 6.27% เมื่อทดสอบด้วยวิธี MTT และความเข้มของ DCF ฟลูออเรสเซนต์ ไม่พบความเป็นพิษจากมะขามป้อมในช่วงความเข้มข้นระหว่าง 20 ถึง 100 มคก./มล. เซลล์กลุ่มที่ได้รับสารสกัดผลมะขามป้อมก่อนเป็นเวลา 48 ชั่วโมง สามารถเพิ่มการอยู่รอด และลดการสร้าง ROS ที่เกิดขึ้นในเซลล์ได้อย่างมีนัยสำคัญ อีกทั้งยังสามารถเหนี่ยวนำการเติมหมู่ฟอสเฟตของ Akt ได้แต่ไม่มีผลเปลี่ยนแปลงวิถี NF-kappaB เมื่อวิเคราะห์ด้วยวิธี western blot analysis ผลการศึกษาชี้ให้เห็นว่าสารสกัดนี้มีประสิทธิภาพในการป้องกันการตายของเซลล์เยื่อบุหลอดเลือดจากไฮโดรเจนเปอร์ออกไซด์ โดยผ่านกลไกการกำจัดสารในกลุ่ม ROS และส่งสัญญาณผ่าน PI3K/Akt อย่างไรก็ตามควรมีการศึกษาเพิ่มเติมในสิ่งมีชีวิตเพื่อสนับสนุนให้มีการพัฒนามะขามป้อมเป็นโภชนเภสัชภัณฑ์ต่อไป