Original Article

Naringin Attenuates Leukocyte Adhesion to Cerebral Endothelium in Type 2 Diabetic Rats

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Background: The increased accumulation of advanced glycation end products (AGEs) under diabetes conditions can promote oxidative stress and inflammation in vasculature and contribute to endothelial dysfunction. Naringin, a flavonoid compound that occurs naturally in citrus fruits, has been shown to have anti-diabetic and anti-oxidant properties.

Objective: To investigate the efficacy of naringin in the improvement of diabetes-induced leukocyte adhesion to cerebral endothelium through AGEs-RAGE-NF-κB pathway.

Materials and Methods: Six-week-old Sprague-Dawley were divided into three groups; normal group (CON: n=8), type 2 diabetes group (DM2: n=8), and type 2 diabetes group with naringin supplementation (DM2-NG: n=8). Rats were fed with high-fat diet for four weeks, followed by a single STZ injection to induce type 2 diabetes. Naringin was supplemented daily by oral gavage feeding (50 mg/kg BW). Twelve weeks after STZ injection with or without naringin supplementation, fasting blood glucose (FBG), serum insulin and calculated homeostatic model assessment of insulin resistance (HOMA-IR) were examined. Leukocyte adhesion at post-capillary venule was carried out by using intravital fluorescence microscopy. AGEs, RAGE and TNF-α were detected by ELISA whereas NF-κB, ICAM-1 were investigated using Western blot analysis and MDA was determined by TBARs assay.

Results: After 12 weeks of naringin feeding into DM2-NG rats, the FBG levels decreased 62.8% compared to those without supplementation. Moreover, the β-cell function was improved by reducing serum insulin levels and HOMA-IR. Not only the endothelial function was improved by reducing the number of leukocyte adhesion, but the expression of ICAM-1 was also decreased. Naringin supplementation also attenuated inflammation and oxidative stress by reducing the levels of AGEs, RAGE, and its downstream molecules, NF-κB-TNF-α.

Conclusion: It is suggested that supplementation with naringin in type 2 diabetes rat model can reduce leukocyte adhesion to vascular endothelium via anti-hyperglycemic, anti-oxidant and anti-inflammatory effects through AGEs-RAGE-NF-κB-TNF-α-ICAM-1 signaling pathway.

Keywords: Type 2 diabetic rats; Naringin; Leukocyte adhesion; Endothelial dysfunction

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It is well recognized that endothelial dysfunction is associated with the microvascular and macrovascular complications in type 2 diabetes mellitus, leading to cerebrovascular disease, cardiovascular disease, retinopathy and nephropathy[1-3]. The endothelial dysfunction is characterized by impairment of vasodilation in response to endothelium-dependent vasodilators, increased vascular inflammation and expression of cell adhesion molecules[4,5]. Accumulating studies reported that hyperglycemia had been noted to promote oxidative stress through the generation of reactive oxygen species (ROS) and inhibition of the anti-oxidant systems[6,7]. Moreover, hyperglycemia-induced oxidative stress is a major inducer to increase the secretion of pro-inflammatory cytokines, leading to promote vascular inflammation[7-9]. In addition, ROS-induced oxidative stress in diabetes are generated by several mechanisms including polyol pathway, diacylglycerol-protein kinase C (DAG/PKC) pathway, hexosamine pathway, and non-enzymatic glycation end product (AGEs) pathway[10,11]. Currently, experimental evidence has highlighted a direct link between excessive ROS and diabetic-induced endothelial dysfunction resulting from the accumulation of AGEs and interaction of AGEs with its receptor, RAGE[12]. AGEs-RAGE interaction induced the inflammatory response, which is an important process in developing vascular dysfunction in various organs[12].

Furthermore, the increased accumulation of AGEs and the AGEs-RAGE interaction stimulates many

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downstream signaling molecules, including transcriptional factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The upregulation of NF-κB triggers the secretion of pro-inflammatory cytokines such as tumor necrotic factor-α (TNF-α), interleukin-1,-6 (IL-1, IL-6) as well as cell adhesion molecules; intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1)[13,14]. In general, leukocytes adhering to endothelium are stimulated in diabetes mellitus associated with elevation of ICAM-1 and VCAM-1 expression[15]. Experimental diabetic studies in an animal model of diabetic retinopathy have revealed that leukocytes adhere to the endothelium, damaged endothelial cells, and increased vascular permeability of retinal vessels[16]. Also, our previous study in STZ-induced diabetic rats demonstrated the enhanced leukocyte adhesion to cerebral endothelium, which is associated with low cerebral blood perfusion. More importantly, the density of leukocyte adhesion could be reduced to near the level of normal control condition by anti-oxidant, vitamin C supplementation[17].

Therefore, the target management for preventing or improving diabetic-induced vascular endothelial has been focused on attenuating oxidative stress and inflammation. Medicinal plants contain bioactive phytochemicals such as alkaloids, sterols, phenolics and flavonoids have been shown to exhibit anti-diabetic, anti-oxidant and anti-inflammation in diabetic management. Naringin (4',5,7-trihydroxy flavanone 7-rhamnoglucoside), is the most abundant flavonoid in grapefruit, but it is hydrolyzed to its corresponding aglycone and sugars in the gastrointestinal tract before absorption[19]. It has been shown to have anti-oxidant, free radical scavenging, anti-inflammatory, and anti-cancer properties[18,19].

With the aim of using medicinal plant, this study was designed to determine the effect of oral naringin supplementation on improving type 2 diabetes-induced vascular endothelial dysfunction, which is characterized by the leukocyte adhesion to cerebral endothelium, and alteration of AGEs-RAGE and its downstream signaling molecules, NF-κB, TNF-α and ICAM-1 expression, in rat brain. The high fat-fed with low STZ-induced type 2 diabetic rat model was conducted to use in our experiments.

Materials and Methods

Animal preparation

Twenty-four male Sprague Dawley rat (SD-rat), 4 to 6 weeks old (weighing 180 to 200 g), were obtained from Nomura Siam International Co, Ltd. Thailand). Throughout the experiment, the rats were housed and maintained under temperature 22±2°C, and 12 hours light-dark cycle. The rats were allowed to acclimatize for one week before the experiment. The experiments were performed in accordance with the Nation Institute of Guidelines for the use of laboratory animals and were approved by the Ethical Committee of Faculty of Medicine, Srinakharinwirot University (certificate number COA/AE-006-2562).

Experimental design

Rats were randomly assigned into three groups (8 rats/group), normal control group (CON), type 2 diabetic group (DM2), and naringin treated DM2 group (DM2-NG). CON group was fed with a regular diet (4.5% fat and 24% protein, with a total calorific value of 3,040 kcal/kg diet) whereas DM2 and DM2-NG groups were fed with high-fat diet (40.0% fat, 35.0% carbohydrate, and 25.0% protein, with a total calorific value of 5,085 kcal/kg diet). In addition, the DM2-NG group receives daily gavage feeding of drinking water-dissolved naringin (purity ≥90% Sigma-Aldrich, USA) at 50 mg/kg BW[20] for 12 weeks after streptozocin induction.

Induction of experimental type 2 diabetic rat model

Type 2 diabetic rat model was induced as described as follows[21]. Rats were fed daily with a high-fat diet for 4 weeks. After 4 weeks of dietary manipulation, rats were injected intravenously (IV) with streptozocin (STZ) at 30 mg/kg body weight in 10 Mm sodium citrate buffer, pH4.5. The normal control rat received a regular diet for 4 weeks followed by 10 Mm sodium citrate buffer (0.5 mL IV). Fasting blood glucose (FBG) was determined on 7 days post-STZ or sodium citrate buffer injection. The rats with a FBG level more than 250 mg/dL were diagnosed as diabetes mellitus and further used for DM2 and DM2-NG groups.

Determination of fasting blood glucose, serum insulin, and HOMA-IR

At week 16th of the experiment, Rats were fasting for 12 hours and blood samples were collected from tail vein to determine fasting blood glucose (FBG) and serum insulin levels by glucostrip AccuCheck® and ELISA technique (Millipore USA) respectively, by following the manufacturer's protocols. The levels of FBG and serum insulin were used to calculate the insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR), according to the following formula: HOMA-IR = (FBG x serum insulin)/405[22], while the unit of FBG is mg/dL and serum insulin is mU/L.

Intravital microscopic observation

On the day of the experiments, the rat was anesthetized with sodium pentobarbital (60 mg/kg BW, ip). The rats were kept warm at 37°C using a warming pad, and a tracheotomy was performed. It was ventilated mechanically with room air and supplemental oxygen. A catheter was inserted into a femoral vein for injection of fluorescence tracer, and a femoral artery was cannulated for arterial blood gas monitoring and maintained within normal limit throughout the experiment (pCO_2 35 to 45 mmHg, pO_2 90 to 100 mmHg, pH 7.35 to 745). A craniotomy was prepared to expose the anterior cerebral cortex, and the dura mater was opened. An artificial cerebrospinal fluid (aCFS) (composition: NaCl = 118.0, KCl = 4.0, $MgSO_4$ = 1.2, CaCl = 1.5, $NaHCO_3$ = 25.0, glucose = 5.0 in mM) was

infused into the cranial space[17]. A fluorescence microscopic system (NIKON, Japan) equipped with a CCD video camera (Hamamutsu Photonics, Japan) was used for intravital observation. As an (20X) objective len was used, the video images were recorded for further analysis.

Evaluation of Leukocyte adhesion

Rhodamine image was used to visualize the behavior of leukocytes in cerebral microvessels. Rhodamine 6 (R6G) (Sigma Aldrich, USA) was dissolved in saline solution at a concentration of 0.3 mg/mL, and 0.3 mL of R6G was administered intravenously. Based on the recorded video image, the number of leukocytes adhering to endothelium were counted in post-capillary venules with a diameter of 20 to 40 mm. When R6G-labeled leukocytes remain stationary longer than 30 seconds in single venue, the number of leukocyte adhesion was expressed as the number of cells per 100 mm post-capillary length[17].

Preparation of rat brain homogenate

At the end of the experiment, the rats were sacrificed with a high dose of sodium pentobarbital (100 mg/kg BW). Thereafter, the brain was removed, and the hippocampal tissue was rapidly separated from the whole brain and then was homogenized in RIPA buffer (Sigma-Aldrich, USA). The homogenate was immediately centrifuged at 12,000 rpm for 20 minutes at 4°C, and the supernatant was collected. The protein concentration in the supernatant was determined by Bio-Rad protein assay (Bio-Rad, CA, USA). The protein samples were stored at -80°C until used for Western blot analysis and ELISA assay.

Determination of NF-κB (p65) subunit and ICAM-1 expression in rat hippocampus

Western blot was used to determine the expression of ICAM-1 and p65 (an active unit of NF-κB) in the homogenate of rat hippocampus. Briefly, the target tissue proteins in the homogenate samples were isolated on 10% SDS-PAGE and transferred to PVDF membranes. After blocking with 5% skim milk, the membrane was incubated with anti-β-actin (Millipore, USA), and anti-p65 antibody (Santa Cruz Biotech, USA) or anti-ICAM -1 antibody (Santa Cruz Biotech, USA), and at 4°C overnight. After that, the specific secondary antibody was added and incubated for 1 hour. The target bands were visualized on the x-ray film by enhanced chemiluminescence (ECL) system. The density of the protein bands was calculated by using the SCION image Program.

Determination of AGEs, RAGE, TNF-α and MDA levels in rat hippocampus

Quantitative sandwich enzyme-linked immunosorbent assay (ELISA) was used to determined the tumor necrosis factor-α (TNF-α) (R&D system, USA), and AGEs and RAGE (Cusabio tech USA) by following the manufacturer's protocols. The level of malondialdehyde was detected by thiobarbituric acid reactive substance (TBARs) assay in the MDA-TBA complex as described in the manufacturer's instruction (Cayman Chemical, USA).

Statistical analysis

All results were presented as mean ± standard error of mean (SEM). The data were collected and analyzed using a one-way analysis of variance (one-way ANOVA) and Tukey's multiple-comparison test were involved after confirmation of normal distributions. Pearson's correlation was used to investigate relationship between number of leukocyte adhesion, ICAM-1 expression and AGEs. The significant difference was considered if the p-value was less than 0.05. Statistical analysis was performed using the GraphPad Prism 5 programs.

Results

The effect of naringin on metabolic parameters in type 2 diabetes

The anti-diabetic effect of naringin was shown in Figure 1. By monitoring FBG, DM2-NG rats showed a significant decrease in FBG level compared to that of DM2 rats (Figure 1A). We found that after 12 weeks of naringin supplementation the FBG of DM2-NG rats was decreased 62.8 % when compared to before supplementation. Besides, naringin also showed the potential to reduce the serum insulin level in type 2 diabetic rats. As shown in Figure 1B, the serum insulin level in DM2-NG rats was significantly decreased if compared to that of DM2 rats ($p<0.001$).

Furthermore, we were also assessing β-cell function and insulin resistance by calculating the homeostatic model assessment of insulin resistance (HOMA-IR) index to confirm the anti-diabetic property of naringin. As expected, we found that HOMA-IR index of DM2-NG rats was ten times lower than those of DM2 rats (Figure 1C). However, all of the parameters in CON rats remained stable throughout the experiment.

The effect of naringin on leukocyte adhesion to post-capillary venule in type2 diabetes

By using fluorecene videomicroscopic visualization, the number of leukocytes adhesion per 100 mm of post-capillary venule length were established. Compared to that of control rats, number of leukocyte adhesion was significantly increased in DM2-rats ($p<0.001$). Interestingly, the post-capillary venules of DM2-NG rats contained a significant reduction of leukocyte adhesion when they were compared to those of DM2 rats ($p<0.001$) (Figure 2A). Moreover, we also investigated the expression of intercellular adhesion molecule-1 (ICAM-1), which plays an essential role in leukocyte adhesion. Obviously, due to the high number of leukocyte adhesion, DM2 rats also showed an increase in ICAM-1 expression in hippocampus homogenate (Figure 2B). Differently, ICAM-1 expression in DM2-NG rats was significantly lower than that of DM2 rats. Thus the expression of ICAM-1 is directly related to the number

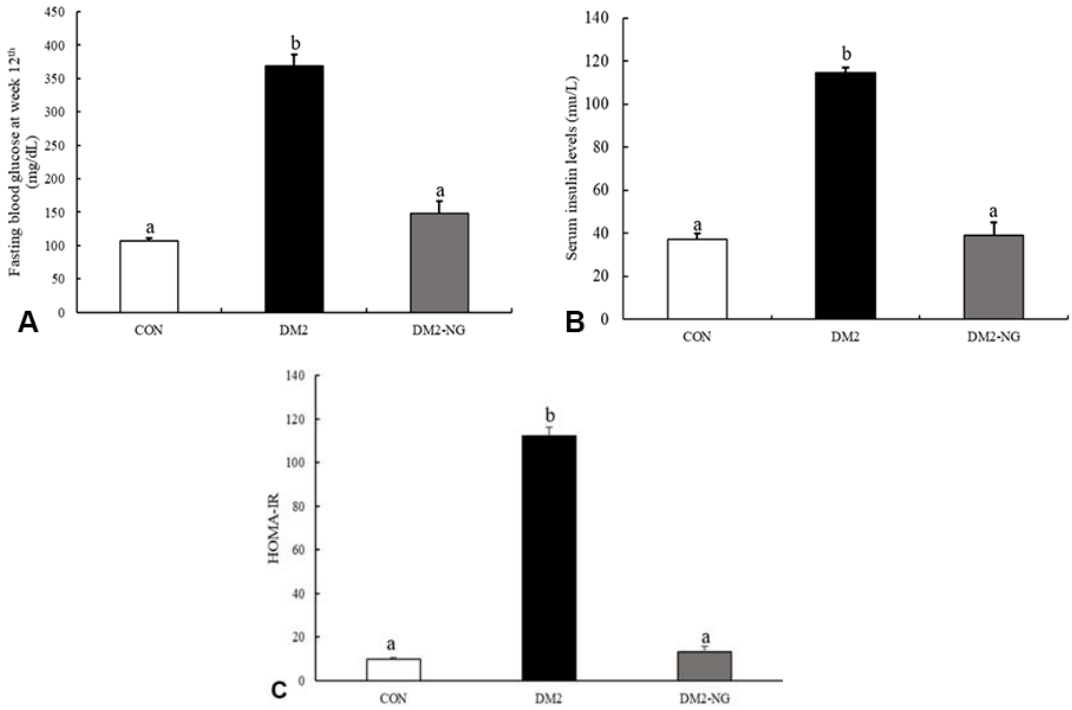

Figure 1. Effect of naringin on type 2 diabetic metabolic parameters: A) Fasting blood glucose level at week 12th, B) serum insulin level, C) homeostatic model assessment of insulin resistance (HOMA-IR). The bar which shares the same superscript symbols are not significantly different. The bar which has different superscript symbols are significantly different ($p<0.001$).

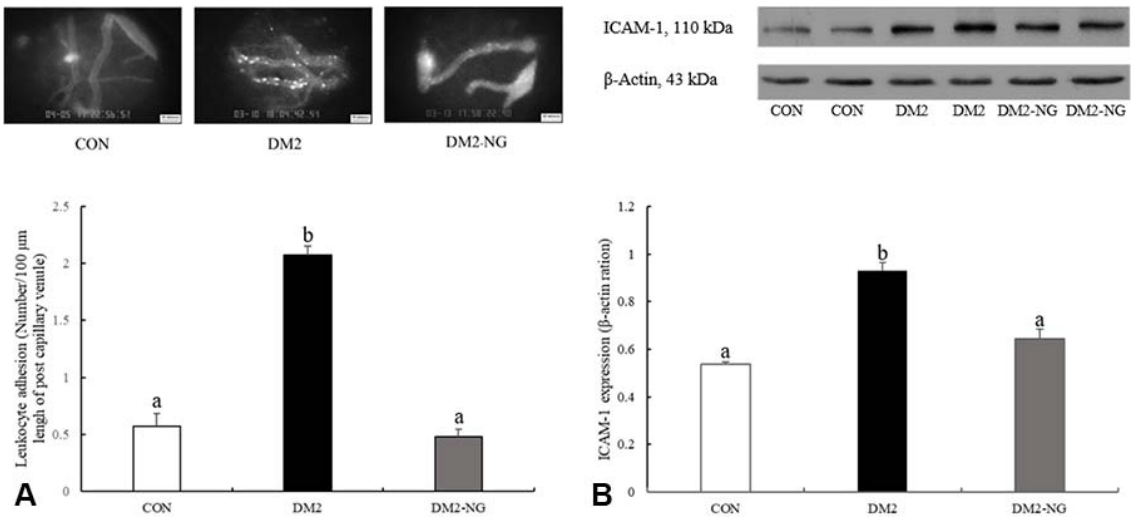

Figure 2. Effect of naringin on leukocyte adhesion: A) number of leukocyte adhesion; B) expression of ICAM-1. The bar which shares the same superscript symbols are not significantly different. The bar which has different superscript symbols are significantly different ($p<0.001$).

of leukocytes adhesion.

The effect of naringin on inflammation and reactive oxygen species (malondyaldehyde: MDA)

Tumor necrosis factor-α (TNF-α) is known as a pro-inflammatory cytokine involved in inflammation. Thus we investigated the level of TNF-α in rat hippocampal tissue by using ELISA. Besides, we also demonstrated MDA levels which indicated the lipid peroxidase in rat hippocampus. The changing of these two parameters went in the same direction. Although a significant increase of their levels was found in DM2 rats, the levels were markedly reduced when supplemented with naringin for 12 weeks (DM2-NG), as shown in Figure 3. These two parameters can be used as indicators of inflammation and oxidative stress.

The effect of naringin on NF-κB (p65), advanced glycation-end product (AGEs), and receptor of advanced glycation end product (RAGE)

To identify the possible mechanism for inflammation and oxidative stress. First, we investigated the levels of AGEs and its receptor (RAGE). As shown in Figure 4A, the AGEs and RAGE levels were significantly increased in DM2 rats compared to that of control rats ($p<0.001$), However, DM2-NG rats showed significantly decreased levels of both AGEs and RAGE when compared to the untreated DM2 group. Additionally, the reaction between AGEs and RAGE may generate signal transduction through the NF-κB pathway. Thus, the expression of p65 was obviously demonstrated in the same direction as the AGEs and RAGE levels. The p65 expression was increased in DM2 rats and decreased in DM2-NG rats (Figure 4B).

Figure 4C and 4D revealed the correlation between leukocyte adhesion, ICAM-1 expression, and AGEs levels which were monitored in CON, DM2, and DM2-NG rats. The Pearson's correlation coefficient (r) of AGEs-Leukocyte adhesion and AGEs-ICAM-1 are 0.864 and 0.833, respectively. These results exhibited strong positive correlations between AGEs levels and leukocyte adhesion which was related to the expression of adhesion molecules.

Discussion

The main finding of the present study was that naringin supplementation attenuated leukocyte adhesion to cerebral endothelium in association with its anti-hyperglycemic, anti-oxidant and anti-inflammatory activities. The results are consistent with previous studies that hyperglycemia, hyperinsulinemia and insulin resistance were developed in HF-STZ-induced type 2 diabetic rat model. Twelve weeks supplementation with naringin into DM2-NG rats showed the reduction of FBG and serum insulin levels. These findings indicated that naringin has the potential to reduce hyperglycemia, hyperinsulinemia as well as improves insulin sensitivity. In 2004, Jung et al suggested that naringin can lower blood glucose by upregulating glucose regulating enzymes such as glucose-6-phosphatase and glucokinase. Moreover, they also found that naringin can increase glycolysis and glycogen concentration in hepatocytes[23]. Therefore, our finding indicated that naringin has the potential to recover metabolic abnormalities in HF-STZ-induced type 2 diabetes as indicated by the decreased hyperglycemia and improved peripheral insulin sensitivity. Unfortunately, we did not investigate the effect of naringin on normal rats, however, in 2012 Xulu et al found that naringin 50 mg/kg B.W. per day did not affect the levels of blood glucose, triglyceride, and serum insulin in normal rat. Besides, they also demonstrated that naringin feeding did not increase the amount of liver enzyme; indicating no side effect of the naringin on normal rats[24].

To examine whether the treatment with naringin could attenuate leukocyte adhesion to cerebral endothelium in type 2 diabetic rats, we experienced direct observation of

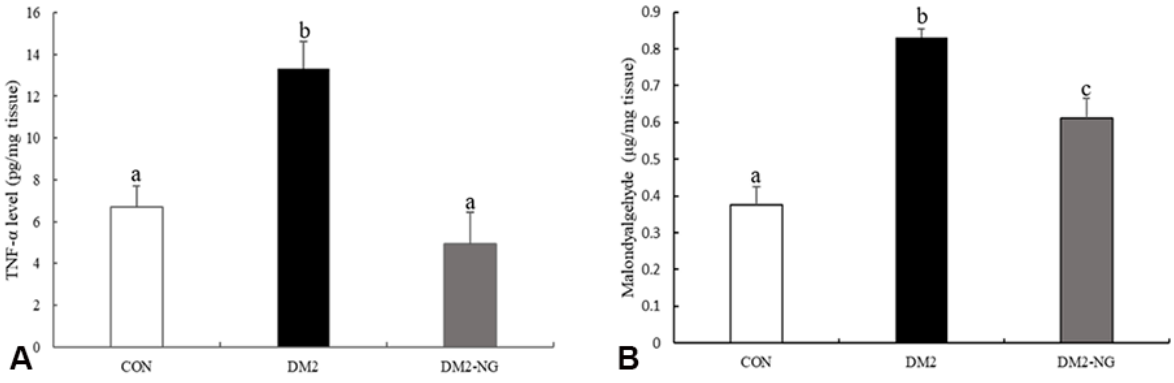

Figure 3. Effect of naringin on inflammation and MDA express: A) level of tumor necrosis factor-α (TNF-α) in rat hippocampal tissue; B) level of malondialdehyde in rat hippocampus tissue. The bar which shares the same superscript symbols are not significantly different. The bar which has different superscript symbols are significantly different ($p<0.05$).

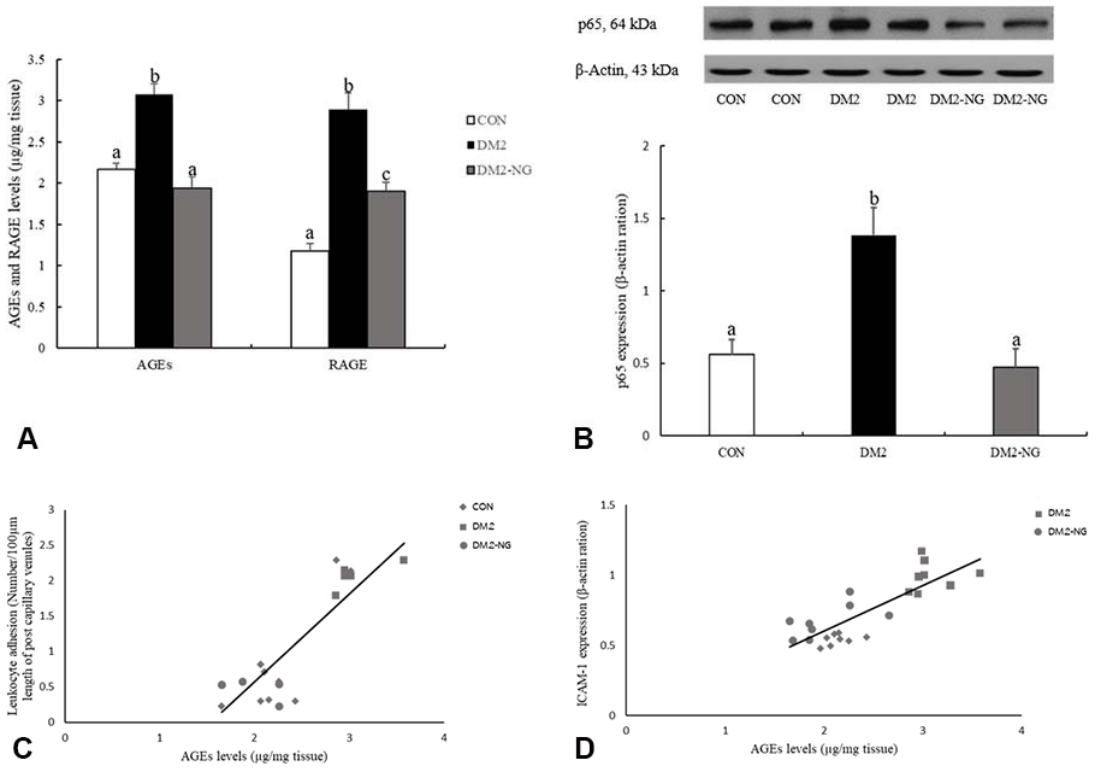

Figure 4. Effect of naringin on molecular activity: A) level of AGEs and RAGE in rat hippocampal tissue; B) expression of p65 in rat hippocampus tissue; C) correlation coefficient between AGEs and leukocyte adhesion which has a correlation (r) of 0.864 ($p<0.001$); D) correlation coefficient between AGEs and ICAM-1 which has a correlation (r) of 0.833, ($p<0.001$). The bar which shares the same superscript symbols are not significantly different. The bar which has different superscript symbols are significantly different ($p<0.05$).

leukocytes behavior in the cerebral post-capillary venule by labeling leukocyte cells with R6G. Under normal conditions, leukocytes do not adhere to endothelial cells. However, in response to inflammation and tissue injury, endothelial cells become activated, and adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), are expressed[25]. Expression of both adhesion molecules on the surface of activated endothelium facilitates the adhesion and transmigration of leukocytes and immune cells, leading to vascular inflammation and endothelial dysfunction[26]. Moreover, the increasing of leukocyte adhesion can induce brain pathology. In 2003 Yuan et al found that reduction of leukocyte adhesion by anti-ICAM-1 antibody can improve vascular permeability and blood-brain barrier (BBB) function in radiation-induced rat[27]. It is known that BBB is an important structure to preserve brain homeostasis and function. Besides, severals studies confirm that reducing leukocyte adhesion decreased infarction size and neural injuries in ischemic/reperfusion model[28-30]. Thus, leukocyte adhesion might play an essential role to induce brain pathology. Our results have shown that the leukocyte adhesion to cerebral endothelium and expression of ICAM-1 were markedly enhanced in DM2 rats. Interestingly, supplementation of naringin in DM2-NG rats could significantly decrease the number of leukocyte adhesion and ICAM-1 expression down to 76.79% and 30.9%, respectively as compared to those of DM2 rats. Several studies demonstrated that hyperglycemia induces activation of endothelial cells by upregulating levels of the adhesion molecules, such as ICAM-1 and VCAM-1[31,32]. In addition, accumulating data have shown that ROS stimulated the expression of cell adhesion molecules on endothelial surfaces, leading to vascular damages[33].

It is known that type 2 diabetes is the major cause to induce endothelial dysfunction through several mechanisms. In 2002, Tan et al found that endothelial dysfunction had a strong relationship with the increased levels of plasma advanced glycation end product (AGEs)[34]. Moreover, the accumulation of AGEs is known as the cause of oxidative stress. In 2010 Guimaraes et al studied the effect of AGEs on hepatic stellate cells and found that ROS levels were increased after administration of hepatic stellate cells with AGEs[35]. AGEs are proteins or lipids that become glycated after exposure to excess glucose[36]. The accumulation

of AGEs in several cell types causes oxidative stress and contributes to microvascular and macrovascular damages by interaction with their receptor, RAGE[37].

In the present study, due to the high level of blood glucose in DM2-rats, we observed the elevation of AGEs and RAGE levels in hippocampal tissues. This is consistent with the study run by McPherson et al which found that AGEs formation was increased in endothelial cells after exposure to hyperglycemic conditions[38]. However, after administering DM2 rat with naringin for 12 weeks, the reduction of AGEs and RAGE levels were observed. Therefore, we suggested that one possible mechanism that naringin can decrease AGEs and RAGE levels is its anti-hyperglycemic property. As previously mentioned, the formation rate of AGEs increased in the hyperglycemic condition; thus, lowering blood glucose levels may decrease the AGEs formation. Due to the elevation of AGEs, we also observed the oxidative stress by determining ROS in the form of lipid peroxidation by product (Malondialdehyde; MDA) using the TBARs assay kit. As shown in Figure 3B, DM2 rats were facing oxidative stress predicted by the high production of MDA in hippocampus tissue.

According to our study, type 2 diabetes can induce oxidative stress through hyperglycemia and the AGEs-RAGE pathway. In addition, naringin supplementation was demonstrated to inhibit ROS production by significant reduction of MDA levels in DM2-NG rats. Thus, our results provide evidence in support of the anti-oxidant property of naringin. The activation of AGEs-RAGE pathway involves the upregulation of transcriptional factor NF-κB, which activates secretion of pro-inflammatory cytokines such as TNF-α[39]. NF-κB also activated the expression of adhesion molecules, including ICAM-1, VCAM-1, and E-selectin[40,41]. From the present experiments, we revealed the increased expression of p65 (the activation site of NF-κB) and TNF-α in hippocampus tissues of DM2 rats. Interestingly, the expression of RAGE, p65, and TNF-α was significantly decreased after naringin supplementation. Moreover, the high correlation between the level of AGEs and the number of leukocytes ($r=0.894$, $p<0.001$) or ICAM-1 expression ($r=0.825$, $p<0.001$) were clearly demonstrated, as shown in Figure 4C and Figure 4D, respectively. These results could confirm the potential effects of naringin supplementation to reduce leukocyte adhesion by attenuation of AGEs-RAGE levels as well as expression of ICAM-1, in DM2-NG rats. In 2017 Ahmed et al demonstrated that naringin at 100 mg/kg BW dosage could decrease lipid peroxidase in type 2 diabetes rats and exhibit the anti-oxidant and anti-diabetic properties[42]. We also study the effect of naringin at a dose of 100 mg/kg BW (data not shown). However, we did not notice the significantly different outcomes between naringin 50 mg/kg BW and 100 mg/kg BW in terms of anti-diabetes, anti-inflammation, and anti-oxidant. Thus, naringin 50 mg/kg BW is an effective dose to study its potential on the type 2 diabetes model. Nevertheless, the study about the dose-dependent of naringin has not been fully understood yet. Therefore, it will be an exemplary aspect of the future study

Apart from our study of naringin, other flavonoids, hesperidin and naringenin, also demonstrates good potential of anti-diabetic, anti-inflammatory, and anti-oxidant properties in type 2 diabetic rat model[20,42]. However, Mahmoud et al did not observe the significant difference results between naringin and hesperidin[20]. Moreover, Ahmed et al also found that naringin and naringenin show the same potential in anti-diabetic, anti-oxidant, and anti-inflammatory properties[42]. Naringin, naringenin and hesperidin are the flavanones found in citrus fruits. Naringin and hesperidin are the citrus flavanone glycoside with the same flavanone structure, but the aglycone of naringin is naringenin whereas aglycone of hesperidin is hesperetin[43].

In summary, the present study demonstrated that supplementation with naringin in type 2 diabetes could reduce leukocyte adhesion to vascular endothelium via anti-hyperglycemic, anti-oxidant and anti-inflammatory effects through AGEs-RAGE-NF-κB-TNF-α-ICAM-1 signaling pathway. Together with our results as well as those of other studies, provide evidences to propose that oral supplementation with naringin might offer a novel food supplement for prevention of cerebral microvascular dysfunction in type 2 diabetes mellitus.

What is already known on this topic?

Naringin has been reported to have pharmacological benefits which are anti-oxidant, antimicrobial, anti-inflammatory, antiapoptotic properties. However, the effects of naringin on cerebral endothelial dysfunction induced by type 2 diabetes have not been reported.

What this study adds?

Oral supplementation of naringin for 12 weeks can reduce leukocyte adhesion to vascular endothelium via anti-hyperglycemic, anti-oxidant and anti-inflammatory effects through AGEs-RAGE-NF-κB-TNF-α-ICAM-1 signaling pathway.

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Potential conflics of interest

The authors declare no conflict of interest.

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