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Anti-inflammatory property of *Parkia speciosa* empty pod extract in human umbilical vein endothelial cells

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ABSTRACT

Parkia speciosa Hassk, locally known as *petai papan*, is a common medicinal plant found in Southeast Asia. Its empty pods were reported to contain high concentrations of polyphenols, particularly quercetin. This study aimed to evaluate the anti-inflammatory properties of *P. speciosa* empty pod extract in human umbilical vein endothelial cells (HUVECs). The empty pods were extracted by sequential fractionation with absolute ethanol and ethyl acetate. HUVECs were divided into four groups. HUVECs were exposed to tumor necrosis factor-α (TNF-α, 10 ng/mL) in the presence (25 µg/mL) or absence of *Parkia speciosa* extract. Quercetin (125 µM) served as the positive control, while another group that was not exposed to TNF-α acted as the negative control. The protein expressions of the inflammatory mediators, which were NF-κB p65, iNOS, COX-2 and VCAM-1, were significantly decreased in the *P. speciosa* and quercetin groups exposed to TNF-α. *P. speciosa* extract and quercetin also significantly reduced intracellular reactive oxygen species and nitric oxide levels as well as inducible nitric oxide synthase (iNOS) activity caused by TNF-α in HUVECs. In conclusion, *P. speciosa* empty pod extract exhibited potential anti-inflammatory properties against TNF-α-induced inflammation, possibly by modulating the NF-κB p65 pathway. The effects were comparable to that of quercetin.

INTRODUCTION

The pathogenesis of many diseases, such as atherosclerosis, hypertension and diabetic vasculopathy, involves inflammation (He *et al*., 2015). Inflammation leads to cellular and tissue injuries. Nuclear factor κB (NF-κB) signaling is believed to be featured in the inflammatory cascade (Hsu *et al*., 2013), resulting in an increase in vascular cell adhesion molecule-1 (VCAM-1) (Conde *et al*., 2017), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression as well as proinflammatory cytokines, like tumor necrosis factor-α (TNF-α) (Hsu *et al*., 2013). Elevated expression of iNOS will increase nitric oxide (NO) and reactive oxygen species production (Hsu *et al*., 2013). TNF-α has been employed in many studies to evoke inflammatory reactions in cultured cells (Cao *et al*., 2009; Qi *et al*., 2010). Many studies have shown that plants with polyphenol compounds possess anti-inflammatory properties (Diaz-Rivas *et al*., 2015; Tuzcu *et al*., 2017). *Parkia speciosa Hassk* or stink bean, a plant indigenous to Southeast Asia and locally known as *petai papan* from the family *Leguminosae*, was reported to contain high levels of polyphenol compounds, particularly in its empty pods (Kamisah *et al*., 2013; Ko *et al*., 2014).

It has been utilized in folk medicine to control hypertension (Lim, 2012). Previously, it was shown that there is prevention of hypertension in rats administered with N(G)-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase (Kamisah *et al*. 2017). Based on this, the current study was conducted to investigate the effects of *P. speciosa* empty pods on TNF-α-induced inflammation in human umbilical vein endothelial cells (HUVECs).

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MATERIALS AND METHODS

Plant material and extraction method

Fresh empty pods of *P. speciosa* were collected from Bidor (Perak, Malaysia) in December 2014. Its voucher specimen (UKMB 40239) was authenticated by a botanist and deposited at the Herbarium of Universiti Kebangsaan Malaysia Bangi, Selangor, Malaysia. De-seeded and dried empty pods were finely chopped and ground to fine powder (1.2 kg) with a blender and then macerated and extracted (every 24 h) in absolute ethanol (3 x 4 L). The filtered extract was evaporated under vacuum using a rotary evaporator. The remaining crude extract was then sequentially partitioned three times with hexane and ethyl acetate. The resultant ethyl acetate extract was utilized for further study based on our preliminary work that showed its highest viability in HUVECs (unpublished data). The yield was 11.261%.

Phytochemical analysis of *P. speciosa* empty pods

High-performance liquid chromatography (HPLC) analysis was conducted to identify the polyphenolic compounds present in the ethyl acetate fractions of *P. speciosa* empty pod ethanolic extract according to the method described by Komolafe *et al*. (2014) with certain modifications. Briefly, the modifications were the use of a chromatographic system (Waters 2535) equipped with a reversed-phased column C-18 (4.6 x 250 mm, 5µm; XBridge, Waters, Dublin, Ireland) and a photodiode array detector (Waters 2998) that was set at 254 and 365 nm. Samples were eluted with 1% aqueous acetic acid solution (A) and acetonitrile (B) at 0. 5 ml/mL with the following gradient: 13% B from 0 to 10 min that was increased to 20%, 30%, 50%, 70% and back to 20% B at 20 min, 30 min, 40 min, 50 min, 60 min, and 80 min, respectively.

All chromatographic procedures were carried out at ambient temperature. The peaks obtained from the elution of the extract which was dissolved in HPLC grade methanol (10 mg/ml) were confirmed by comparing the retention time with those of reference standards (gallic acid, ellagic acid, catechin and quercetin).

Cell viability assay

HUVECs (Gibco, Grand Island, NY, USA) were grown in endothelial cell media and maintained in 1% endothelial cell growth supplement according to the manufacturer's recommended protocol. Cells were passaged at confluence and only culture passages 3 to 6 were made use of in this study. 3-(4,5-Dimethylthiazol-2-yl)-5- (3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was performed with the CellTiter 96 Aqueous One Solution Proliferation Assay Kit (Promega, Madison, USA) to measure cell viability.

Cell culture treatment

HUVECs were pretreated with *P. speciosa* (25 µg/mL) extract or quercetin (125 µM) (Indra *et al*., 2013) for 6 h before being exposed to TNF-α (10 ng/mL; R&D Systems, Abingdon, UK) and actinomycin D (1µg/mL; Sigma-Aldrich, USA) (Zhou-Stache *et al*., 2002) for another 16 h. Quercetin served as the positive control. Another group without any treatment served as the negative control group.

Western immunoblot analysis

The expression of targeted proteins, which were NF-kB p65 (Santa Cruz Biotech, USA), iNOS (Abcam, USA), COX-2 (Cell Signaling, USA), VCAM-1 (Santa Cruz Biotech, USA), and β-actin (Santa Cruz Biotech, USA) were determined by Western blot analysis with the respective primary antibodies (1:1000) and secondary antibodies (1:3000) (Xie *et al*., 2000). Incubation with β-actin antibody was carried out as a comparative control.

Measurement of intracellular ROS and NO levels as well as iNOS activity

Intracellular ROS levels were measured via a fluorescent probe, 2′,7′ dichlorodihydrofluorescein (DCFH-DA, Sigma Co., USA) (Wen *et al*., 2013). NO levels in the cell culture medium were measured with Griess reagent (Miranda *et al*., 2001). Meanwhile, the activity of iNOS was determined according to the manufacturer's assay kit protocol (Elabscience, China).

Statistical analysis

All experiments were carried out in triplicate. The data were expressed as mean ± standard error of mean (SEM). Normally distributed data were statistically analyzed through one way analysis of variance (ANOVA) followed by Tukey's post-hoc test, while non-normally distributed data was evaluated by the Kruskal-Wallis test. P values < 0.05 were considered significant.

RESULTS

Phytochemical analysis

Only two standards (catechin and quercetin) appeared when eluted at 365 nm. There were six major peaks that appeared in the chromatogram of the extract. However, when compared with the reference standards, only quercetin was identified (Figure 1). All peaks of the reference standards (gallic acid, ellagic acid, catechin, and quercetin) appeared at 254 nm, but no peaks appeared in the sample (data not shown). The quercetin content in the extract was 5.84 mg per 100 g dry extract.

Viability percentage

TNF-α significantly reduced cell viability by approximately 50% when compared to the negative control (without TNF-α or extract). Preincubation with the extract at 12 µg/ml and larger concentrations significantly increased cell viability in HUVECs exposed to TNF-α. However, no difference was noted among the groups given the extract (Figure 2).

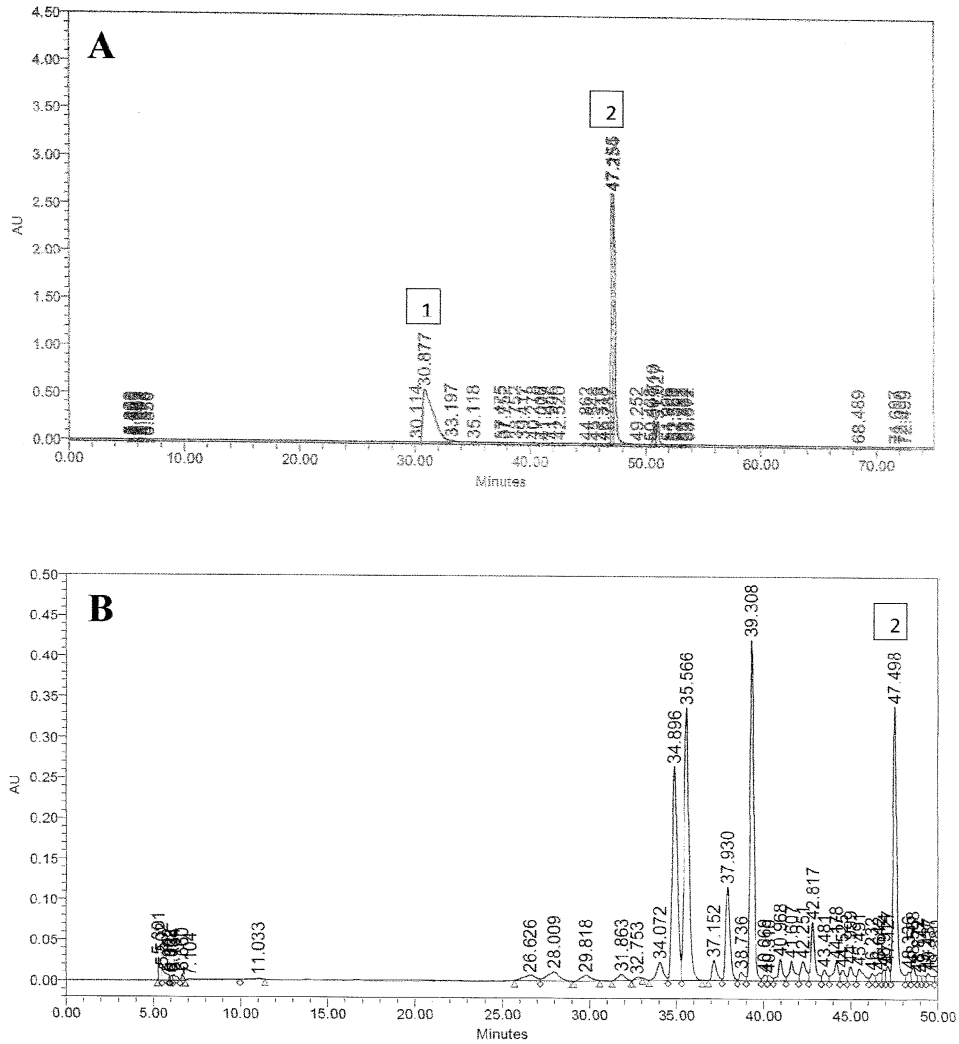

Fig. 1: Chromatogram of reference standards (A) (peak 1: catechin; peak 2: quercetin) and parkia speciosa extract (B) (peak 2: quercetin) at 365 nm.

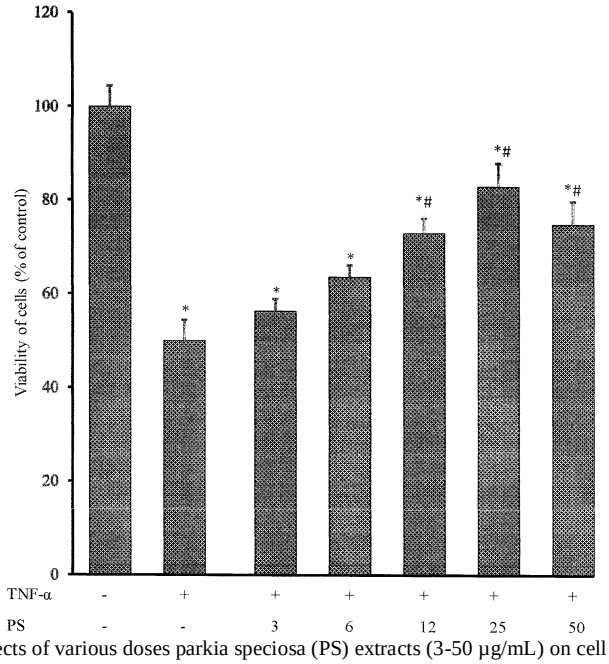

Fig. 2: effects of various doses parkia speciosa (PS) extracts (3-50 µg/mL) on cell viability using MTS assay after being exposed to TNF-α Bars represent mean ± SEM (n =3), *P < 0.05.

Protein expressions of NF-κB p65, iNOS, COX-2, and VCAM-1

Stimulation with TNF-α elevated NF-κB p65 protein expression in HUVECs compared to the negative control. *P. speciosa* and quercetin treatments significantly reduced NF-κB p65 protein expression versus the TNF-α-induced group. The expression of NF-κB p65 in the *P. speciosa* group was significantly higher than the negative control and quercetin groups (Figure 3A). Further, TNF-α incubation rose iNOS protein expression in HUVECs. With this, both *P. speciosa* and quercetin groups had significantly lower iNOS protein expression in cells exposed to TNF-α than the TNF-α alone group (Figure 3B). The protein expression of COX-2 in HUVECs was significantly elevated in cells incubated with TNF-α compared to the negative control. Pretreatments of *P. speciosa* and quercetin significantly mitigated the effect of TNF-α on COX-2 protein expression. The *P. speciosa* group, but not the quercetin group, had significantly higher COX-2 protein expression than the negative control. The expression of the extract group was significantly greater than the quercetin group (Figure 4A).

VCAM-1 protein expression was significantly elevated in TNF-α-induced group compared to the negative control. The expression of VCAM-1 in both treated groups (extract and quercetin) was significantly reduced. Moreover, no difference was evident between the extract and quercetin groups (Figure 4B).

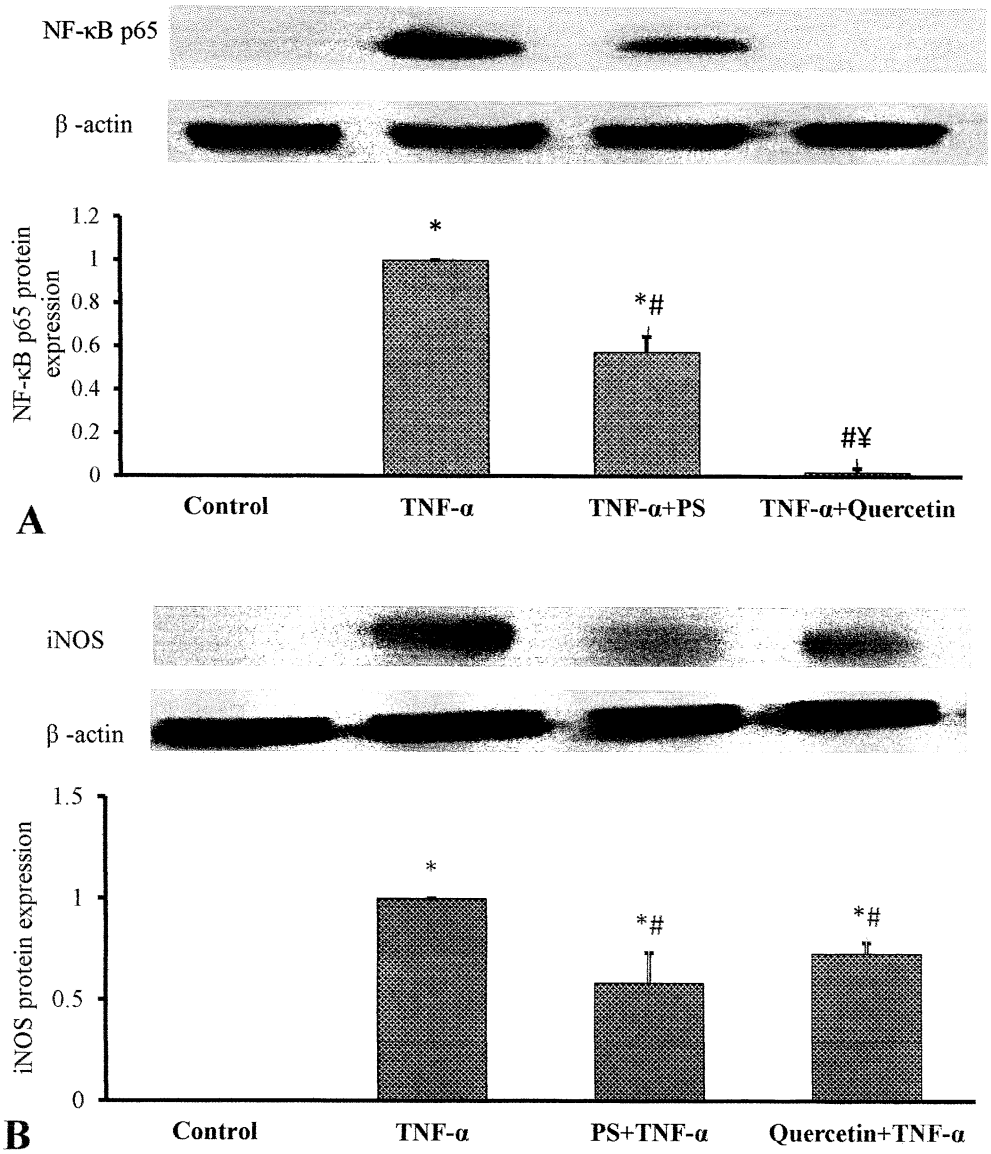

Fig. 3: Effects of parkia speciosa extract (PS, 25 µg/mL) and quercetin (125 µM) on NF – Kb (A) and inducible nitric oxide synthase (Inos) (B) protein expression in TNF-α-activated (10 ng/ml) HUVECs. The data were expressed as means ± SEM (n=3), *P <0.05 vs control, #P <0.05 vs TNF-α.

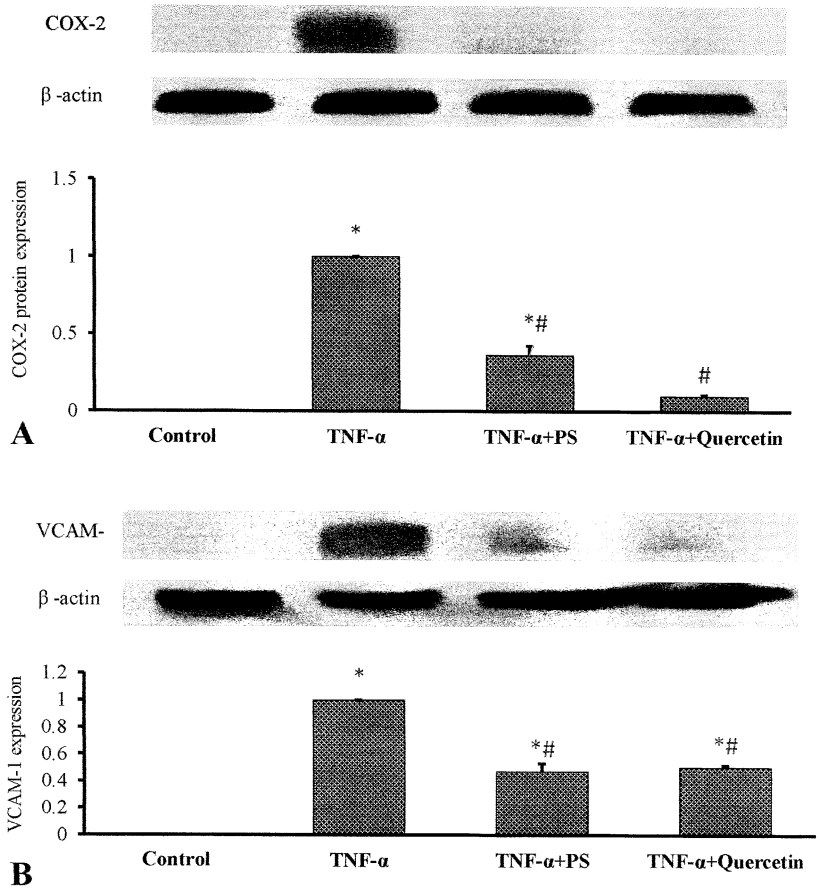

Fig. 4: Effects of parkia speciosa extract (PS, 25 µg/mL) querectin (125 µM) on COX-2(A) and VCAM-1 (B) protein expression in TNF-α-activated (10 ng/ml) HUVECs. The data were expressed as means ± SEM (n=3) *P<0.05 vs #P<0.05 vs TNF-α

Table 1: Inflammatory parameters measured in HUVECs pretreated with *P. speciosa* (PS, 25 µg/ml) or quercetin (125 µM) and exposed to TNF-α (10 ng/ml).

	Control	TNF-α	TNF-α + PS	TNF-α + Quercetin
iNOS activity (ng/ml)	0.23 ± 0.03	3.40 ± 0.10*	2.68 ± 0.02*#	2.24 ± 0.30*#
NO level (µM)	3.16 ± 0.17	17.53 ± 0.16*	10.24 ± 0.25*#	6.83 ± 0.20*#
ROS level (% of control)	100.0 ± 0.0	182.2 ± 1.7*	120.0 ± 5.4#	97.7 ± 6.9#

*Different from control (*P*< 0.05); #different from TNF-α group (*P*< 0.05).

Intracellular iNOS activity, NO and ROS levels

Exposure to TNF-α significantly elevated iNOS activity and NO levels (*P*< 0.05). Pretreatments of *P. speciosa* extract and quercetin similarly diminished the elevation of both parameters. HUVECs treated with TNF-α had significantly higher levels of intracellular ROS than the negative control. Pretreatment of the extract or quercetin significantly reversed the effect of TNF-α on intracellular ROS levels. No significant differences with regards to ROS levels were observed among the extract, quercetin, and negative control groups (Table 1).

DISCUSSION

Chromatographic analysis showed there to be the presence of quercetin in the extract. Ko *et al*. (2014) also reported the components of the phytochemical present in the *Parkia speciosa* empty pod extract. We attempted to analyze the phytochemical content in the extract via their method but failed.

They had described the presence of gallic acid, catechin, chlorogenic acid, quercetin, ellagic acid, kaempferol, vanillic acid, and epicatechinwithin their extract. Site and time of collection (season) could affect the composition of phytochemicals in plants. In particular, they analyzed ethanol extract without further fractionation through HPLC while in our study, we analyzed the ethyl acetate fraction of the ethanolic extract. This might explain the discrepancy in our findings. A recent study also demonstrated the presence of quercetin in *Parkia speciosa* empty pod methanol extract (Kamisah *et al*., 2017).

This study was a preliminary investigation with the objective of screening the anti-inflammatory of the *P. speciosa* empty pod extract in HUVECs. To the best of our knowledge, there is no single study that has reported the anti-inflammatory properties of this plant. The concentration of *P. speciosa* extract at 25 µg/ml was chosen based on its highest cell viability in HUVECs co-incubated with TNF-α and the use of quercetin as the positive control was because of its presence in the extract. TNF-α

increased NF-κB p65 protein expression in HUVECs as similarly reported in other studies (Cao *et al*., 2009; Profumo *et al*., 2016). NF-κB protein complex is involved in several cellular responses to stimuli, such as free radicals, stress, and cytokines (Donato *et al*., 2015), associated with activation of adhesion molecules, chemokines, and cytokines (Valen *et al*., 2001). Exposure of cells to TNF-α causes phosphorylation of the NF-κB inhibitory protein, IκB, which mediates its translocation to the nucleus and regulates the expression of numerous genes and proteins involved in inflammation (Lawrence, 2009). Activation of the NF-κB pathway activates the gene that encodes iNOS protein (Aktan, 2004), which is observed as enhanced iNOS protein expression and activity leading to increased NO production as per our study. *P. speciosa* and quercetin pretreatments had significantly decreased these parameters. The protective effects of the extract were most likely attributable to its polyphenolic content, especially that of quercetin. The effects of quercetin seemed to be more prominent earlier in the pathway (NF-κB) than at the later stages (iNOS). Quercetin has been described to inhibit NF-κB (Indra *et al*., 2013) and iNOS expression (Garcia-Mediavilla *et al*., 2007).

Activation of iNOS would further elevate NO (Sarath *et al*., 2014) and ROS production (Cook, 2006) in TNF-α-induced cells as similarly observed during the state of stress (Kamisah *et al*., 2016). Increased production of NO may couple with ROS in the cells to form peroxynitrite radicals (Cook, 2006), which promote further damage to these cells. Both *P. speciosa* and quercetin reduced TNF-α-induced NO levels but inhibited ROS production. The greater impact of both pretreatments on ROS could be also attributed to their potent antioxidant properties (Kamisah *et al*., 2013; Zhu *et al*., 2017) in addition to their anti-inflammatory properties. Our recent study indicated that *in vivo* supplementation of *P. speciosa* reduced NADPH oxidase enzyme in hypertensive rats (Kamisah *et al*., 2017). This enzyme is a source of free radicals, especially superoxide anion (Klima *et al*., 2013). Quercetin was also reported to attenuate NADPH oxidase expression (Xiao *et al*., 2017).

COX-2 protein expression is upregulated in TNF-α-induced cells. COX-2 is an enzyme for prostaglandin synthesis that is responsible in inflammation (Li *et al*., 2014). Upregulation of the enzyme by NF-κB is partially responsible for inflammatory responses (Hsu *et al*., 2013). The extract and quercetin in our study exhibited decreasing the expression of NF-kB-associated downstream inducible enzymes, like iNOS and COX-2, as well as via upstream mechanisms of NF-κB by diminishing ROS and NO production (Hsu *et al*., 2013). Quercetin was demonstrated to inhibit COX-2 expression (Garcia-Mediavilla *et al*., 2007).

NO may also modulate endothelial cell adhesion molecule expression in cultured endothelial cells *in vitro* by various mechanisms. The upregulated expression of cell adhesion molecules on endothelial cells will alter the adhesive properties of vasculature, which implies a signal for HUVECs activation (Krieglstein and Granger, 2001). VCAM-1 is constitutively expressed in the vascular endothelium when induced by proinflammatory cytokines (Qi *et al*., 2010). In the current study, VCAM-1 expression was downregulated by *P. speciosa* and quercetin in TNF-α-induced HUVECs, which was consistent with previous work (Bhaskar *et al*., 2016). VCAM-1 plays an important role in the early development of atherosclerosis (Bhaskar *et al*., 2016). Therefore, our findings suggest that *P. speciosa* extract may be beneficial for attenuating early progression of atherosclerosis. Anti-inflammatory properties of *P. speciosa* extract in the work presented here could be further explored in future studies in diseased states.

CONCLUSION

Parkia speciosa empty pod extract treatments attenuate TNF-α-induced inflammatory responses in HUVECs by blocking the activation of NF-κB p65, which leads to a reduction in iNOS, COX-2, and VCAM-1 expression as well as ROS and NO production. The effects were comparable to that of quercetin.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aktan F. iNOS-mediated nitric oxide production and its regulation. Life Sci., 2004; 75:639-653.

Bhaskar S, Sudhakaran PR, Helen A. Quercetin attenuates atherosclerotic inflammation and adhesion molecule expression by modulating TLR-NF-κB signaling pathway. Cell Immunol., 2016; 310:131-140.

Cao LH, Lee YJ, Kang DG, Kim JS, Lee HS. Effect *of Zanthoxylum schinifolium* on TNF-α-induced vascular inflammation in human umbilical vein endothelial cells. Vascul. Pharmacol., 2009; 50:200-207.

Conde E, Giménez-Moyano S, Martín-Gómez L, Rodríguez M, Ramos ME, Aguado-Fraile E, Blanco-Sanchez I, Saiz A, García-Bermejo ML. HIF-1α induction during reperfusion avoids maladaptive repair after renal ischemia/reperfusion involving miR127-3p. Sci. Rep., 2017; 7: 41099.

Cook S. Coronary artery disease, nitric oxide and oxidative stress: the "Yin-Yang" effect--a Chinese concept for a worldwide pandemic. Swiss Med. Wkly., 2006; 136:103-113.

Díaz-Rivas JO, Herrera-Carrera E, Gallegos-Infante JA, Rocha-Guzmán NE, González-Laredo RF, Moreno-Jiménez MR, Ramos-Gómez M, Reynoso-Camacho R, Larrosa-Pérez M, Gallegos-Corona MA. Gastroprotective potential of *Buddleja scordioides* Kunth Scrophulariaceae infusions; effects into the modulation of antioxidant enzymes and inflammation markers in an in vivo model. J. Ethnopharmacol., 2015; 169:280-286.

Donato AJ, Morgan RG, Walker AE, Lesniewski LA. Cellular and molecular biology of aging endothelial cells. J. Mol. Cell. Cardiol., 2015; 89:122-135.

García-Mediavilla V, Crespo I, Collado PS, Esteller A, Sánchez-Campos S, Tuñón MJ, González-Gallego J. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang liver cells. Eur. J. Pharmacol., 2007; 557:221-229.

He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: how are they linked? Molecules, 2015; 20:9183-9213.

Hsu CC, Lien JC, Chang CW, Chang CH, Kuo SC, Huang TF. Yuwen02f1 suppresses LPS-induced endotoxemia and adjuvant-induced arthritis primarily through blockade of ROS formation, NFkB and MAPK activation. Biochem. Pharmacol., 2013; 85:385-395.

Indra MR, Karyono S, Ratnawati R, Malik SG. Quercetin suppresses inflammation by reducing ERK1/2 phosphorylation and NF kappa B activation in leptin-induced human umbilical vein endothelial cells (HUVECs). BMC Res. Notes, 2013; 6:275.

Kamisah Y, Ang SM, Othman F, Nurul-Iman BS, Qodriyah HM. Renoprotective effect of virgin coconut oil in heated palm oil diet-induced hypertensive rats. Appl. Physiol. Nutr. Metab,,2016; 41:1033-1038.

Kamisah Y, Othman F, Qodriyah HMS, Jaarin K. *Parkia speciosaHassk.*: A potential phytomedicine. Evid. Based Complement. Alternat Med., 2013; 2013:709028.

Kamisah Y, Zuhair JSF, Juliana AH, Jaarin K. *Parkia speciosa* empty pod prevents hypertension and cardiac damage in rats given N(G)-nitro-l-arginine methyl ester. Biomed. Pharmacother., 2017; 96:291-298.

Klima L, Kawecka-Jaszcz K, Stolarz-Skrzypek K, Menne J, Fijorek K, Olszanecka A, Wojciechowska W, Bilo G, Czarnecka D. Structure and function of large arteries in hypertension in relation to oxidative stress markers. Kardiol. Pol., 2013; 71:917-923.

Ko HJ, Ang LH, Ng LT. Antioxidant activities and polyphenolic constituents of bitter bean *Parkia speciosa*. Int. J. Food Prop., 2014; 17:1977-1986.

Komolafe K, Olaleye TM, Omotuyi OI, Boligon AA, Athayde ML, Akindahunsi AA, Teixeira da Rocha JB. In vitro antioxidant activity and effect of *Parkiabiglobosa* bark extract on mitochondrial redox status. J. Acupunct. Meridian Stud., 2014; 7:202-210.

Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular diseases. Am. J. Hypertens., 2001; 14:44S-54S.

Lawrence T. The nuclear factor NF-kappa B pathway in inflammation. Cold Spring Harb. Perspect. Biol., 2009; 1:a001651.

Li L, Wang L, Wu Z, Yao L, Wu Y, Huang L, Liu K, Zhou X, Gou D. Anthocyanin-rich fractions from red raspberries attenuate inflammation in both RAW264. 7 macrophages and a mouse model of colitis. Sci. Rep., 2014; 4:6234.

Lim TK. 2012. *Parkia speciosa*, In: Edible Medicinal and Non-Medicinal Plants, Volume 2 (Fruits), Netherlands: Springer 798-803.

Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 2001; 5:62–71.

Profumo E, Buttari B, D'Arcangelo D, Tinaburri L, Dettori MA, Fabbri D, Delogu G, Riganò R. The nutraceutical dehydrozingerone and its dimer counteract inflammation- and oxidative stress-induced dysfunction of in vitro cultured human endothelial cells: a novel perspective for the prevention and therapy of atherosclerosis. Oxid. Med. Cell. Longev., 2016; 2016:1246485.

Qi Y, Liang J, She ZG, Cai Y, Wang J, Lei T, Stallcup WB, Fu M. MCP-induced protein 1 suppresses TNFα-induced VCAM-1 expression in human endothelial cells. FEBS Lett., 2010; 584:3065-3072.

Sarath TS, Waghe P, Gupta P, Choudhury S, Kannan K, Pillai AH, Harikumar SK, Mishra SK, Sarkar SN. Atorvastatin ameliorates arsenic-induced hypertension and enhancement of vascular redox signaling in rats. Toxicol. Appl. Pharmacol., 2014; 280:443-454.

Tuzcu Z, Orhan C, Sahin N, Juturu V, Sahin K. Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rats. Oxid. Med. Cell. Longev., 2017; 2017:1583098.

Valen G, Yan ZQ, Hansson GK. Nuclear factor kappa-B and the heart. J. Am. Coll. Cardiol., 2001; 38:307-314.

Wen YD, Wang H, Kho SH, Rinkiko S, Sheng X, Shen HM, Zhu YZ. Hydrogen sulfide protects HUVECs against hydrogen peroxide induced mitochondrial dysfunction and oxidative stress. PLoS One, 2013; 8:e53147.

Xiao L, Liu L, Guo X, Zhang S, Wang J, Zhou F, Liu L, Tang Y, Yao P. Quercetin attenuates high fat diet-induced atherosclerosis in apolipoprotein E knockout mice: A critical role of NADPH oxidase. Food Chem. Toxicol., 2017; 105:22-33.

Xie C, Reusse A, Dai J, Zay K, Harnett J, Churg A. TNF-alpha increases tracheal epithelial asbestos and fiberglass binding via a NF-kappa B-dependent mechanism. Am. J. Physiol. Lung Cell. Mol. Physiol., 2000; 279:L608-L614.

Zhou-Stache J, Buettner R, Artmann G, Mittermayer C, Bosserhoff AK. Inhibition of TNF-alpha induced cell death in human umbilical vein endothelial cells and Jurkat cells by protocatechuic acid. Med. Biol. Eng. Comput., 2002; 40:698-703.

Zhu X, Li N, Wang Y, Ding L, Chen H, Yu Y, Shi X. Protective effects of quercetin on UVB irradiation‐induced cytotoxicity through ROS clearance in keratinocyte cells. Oncol. Rep., 2017; 37:209-218.

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