Fimbristylis ovata and *Artemisia vulgaris* extracts exhibited an inhibitory effect against AGEs-mediated RAGE expression, ROS generation, and inflammation in THP-1 cells

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Abstract

Advanced glycation end products (AGEs) can induce inflammatory signaling pathways through the receptor for AGEs (RAGE). Targeting RAGE could be a therapeutic strategy for treating chronic inflammation mediated by the AGE-RAGE axis. This study aimed to investigate the effects of *Fimbristylis ovata* and *Artemisia vulgaris* extracts on AGE-RAGE signaling and AGE-mediated oxidative stress and inflammation in THP-1 cells. *F. ovata* and *A. vulgaris* were extracted by a Soxhlet extraction, and antioxidant capacity was evaluated using DPPH and ABTS assays. The human monocytic cell line THP-1 was treated with AGE (600 μg/ml) with and without *F. ovata* and *A. vulgaris* extracts (100 μg/ml). The mitochondria-targeting antioxidant MitoQ (2 μg/ml) was used as a positive control. Cell viability, ROS generation, RAGE, AGE-RAGE signaling pathway components, and inflammatory cytokine levels were analyzed. *F. ovata* and *A. vulgaris* extracts showed antioxidative effects in non-cell-based assays. Treatment of THP-1 cells with AGE significantly increased the protein levels of RAGE and significantly increased the mRNA expression of cytokines, including TNF-α, IL-1β, and IL-6. AGEs induced the generation of ROS and levels of signaling molecules downstream of RAGE, including phosphorylated and total Erk1/2, JNK, and p38 MAPK, although not significantly. *F. ovata* and *A. vulgaris* extracts significantly decreased the protein levels of RAGE and significantly decreased the mRNA levels of cytokines. In conclusion, this study revealed that *F. ovata* and *A. vulgaris* extracts exert anti-inflammatory effects through the AGE-RAGE axis. However, details on this anti-inflammatory effect through AGE-RAGE signaling should be further investigated.

Keywords: AGEs-RAGE signaling, Oxidative stress, Inflammation, *Fimbristylis ovata*, *Artemisia vulgaris*, MitoQ.

Background

Advanced glycation end products (AGEs) are heterogeneous molecules formed by nonenzymatic glycation and protein, lipid and nucleic acid oxidation. In biological systems, the process of endogenous AGE formation and accumulation in various tissues begins under diabetic hyperglycemia and oxidative stress conditions. In addition to endogenously produced AGEs, AGEs also exist in heat-processed foods and cigarette smoke [1,2,3,4]. AGE-induced inflammation has been recognized as a key mechanism underlying chronic diseases (e.g., atherosclerosis), as AGEs can activate several inflammatory signaling pathways by binding to their receptor, receptor for AGEs (RAGE), and regulate the release of inflammatory molecules through oxidative stress [5, 6]. A large number of studies have reported that the AGE-RAGE interaction leads to an increase in oxidative stress and to the activation of various cell signaling pathways, including mitogen-activated protein kinases (MAPKs), nuclear factor-kappa B (NF-κB), and phosphoinositide 3-kinase (PI3K)-Akt, which lead to the expression of inflammation-related genes and promote inflammation [7,8,9,10].

Increasing evidence has suggested that reactive oxygen species (ROS) affect the biosynthesis of inflammatory modulators at the transcriptional level by modulating redox-sensitive transcription factors, including NF-κB, Nrf2, and AP-1 [11]. ROS also play a role in promoting inflammation through MAPK signaling cascades, including extracellular signal-regulated protein kinase (ERK), p38MAPK, and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), which regulate the activity of downstream transcription factors (e.g., NF-κB, ATF-2, and AP-1) and lead to the increased production of numerous inflammatory mediators, growth factors, and proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [12, 13].

A number of natural and synthetic antioxidative compounds have been mentioned as therapeutic strategies for the treatment of many pathophysiological conditions and oxidative stress-related inflammatory diseases [14]. Phytochemicals, a group of chemicals derived from many kinds of fruits and plants, have long been highlighted due to their benefits for human health and their pharmacological activities under several pathological conditions [15]. Numerous polyphenolic compounds and extracts of polyphenolic-rich plants possess antioxidant, anticancer, and anti-inflammatory properties [14]. An in vitro study has shown that flavonoids have therapeutic effects on complications of diabetes due to their antioxidant effects against oxidative stress mediated by AGEs [16]. It has been suggested that active flavonoid derivatives in herbs exhibit potent anti-inflammatory activity [17]. Traditional Thai herbal therapies may be an alternative treatment option for inflammation-related diseases, such as type 2 diabetes [18], cardiovascular diseases [19], rheumatoid arthritis [20], chronic inflammatory lung disease [21], asthma [22], and Alzheimer's disease [23]. *Fimbristylis ovata* (*Burm.f.*) *Kern* (*F. ovata*) is a plant in the Cyperaceae family [24]. Previous studies reported that plants in the Cyperaceae family contain several antioxidant components and have anti-inflammatory properties [25,26,27], antipyretic effects, antinociceptive effects, and activity against *Aedes* mosquito species [28, 29]. *F. ovata* is traditionally used to treat various diseases [30, 31]. In our previous study, we have shown that *F. ovata* has antioxidant activity, anti-inflammatory properties, and neuroprotective potential. Moreover, chemical analysis of *F. ovata* extracts revealed potential active phytochemical compounds with neuroprotective substances [32, 33]. *Artemisia vulgaris L. var. indica Maxim* (*A. vulgaris*) belongs to the Compositae family. There is evidence that *A. vulgaris* contains several polyphenolic compounds [34]. Previous studies reported that *A. vulgaris* has therapeutic properties such as antimalarial, antioxidant, anti-inflammatory, and anticancer properties [35, 36]. However, no study has examined the antioxidant and anti-inflammatory effects of *F. ovata* and *A. vulgaris* extracts prepared with different conventional methods. Therefore, this study aimed to investigate whether *F. ovata* and *A. vulgaris*

extracts can suppress AGE-RAGE signaling activation-induced inflammatory responses and oxidative stress in THP-1 monocytes. Discoveries from this study could help us to better understand the mechanisms of each extract separated by sequential extraction, which allows natural products to be divided according to their polarity in extraction solvents. We expect that these results will provide insights into the roles of these extracts in inflammatory conditions, particularly those caused by AGEs.

Methods

Preparation of plant extracts

F. ovata and *A. vulgaris* were identified by Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand (voucher No. 013431 (BCU) and A015134 (BCU), respectively). The fresh plants were cleaned with distilled water and then oven-dried at 45 °C for 5 days. Dried plants were ground to powder and extracted with petroleum ether, dichloromethane, and methanol 1:10 (w/v) by a Soxhlet extractor. The extracts were filtered, and the solvent was evaporated. Dimethyl sulfoxide (DMSO) was used to dissolve the plant crude extract to establish the 100 mg/ml stock solution. Stock solutions were stored protected from light at − 20 °C.

DPPH assay

Antioxidant capacity was investigated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method based on electron transfer between DPPH and the antioxidants in the plant extracts. Briefly, a calibration curve of ascorbic acid was prepared. Plant extracts (20 µl) were added to DPPH reagent (180 µl) in a 96-well plate and then incubated in the dark for 30 min. The absorbance at 517 nm was measured using a microplate reader (BioTek, VT, USA). The antioxidant activity was reported as mg vitamin C equivalent antioxidant capacity (VCEAC)/g of dried plant.

ABTS assay

Antioxidant activity was analyzed by the reaction between the plant extracts and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) cation radical (ABTS• +). Briefly, a fresh ABTS• + solution was prepared by reacting ABTS reagent with potassium persulfate. A calibration curve of ascorbic acid was prepared. The plant extracts (20 µl) were added to working reagent (180 µl) in a 96-well plate and then incubated in the dark for 45 min. The absorbance at 734 nm was measured. The antioxidant activity was reported as mg VCEAC/g of dried plant.

Cell culture

The human monocytic cell line THP-1 was grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified incubator with 5% CO_2 at 37 °C.

MTS assay

Cell viability was investigated by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, which measures the mitochondrial enzyme activity that reduces MTS to a formazan product that is soluble in the tissue culture medium. Briefly, THP-1 cells were seeded in a 96-well plate at a density of 5×10^5 cells/ml and differentiated into macrophages by

stimulation with 10 ng/ml phorbol 12-myristate 13-acetate. Macrophages were then treated with either plant extracts or AGE-BSA for 24 h. Thereafter, MTS was added and incubated in a humidified incubator with 5% CO_2 at 37 °C for 4 h. The absorbance at 490 nm was measured using a microplate reader. Cells without treatment were used as a negative control. Cell viability was calculated according to the following.

formula: % cell viability = [(absorbance of the treatment group – blank) × 100/(absorbance of the control group – blank)].

Determination of intracellular ROS generation

The percentage of cells undergoing oxidative stress based on the detection of intracellular superoxide radicals was analyzed using a Muse® Oxidative Stress kit (Merck, Darmstadt, Germany). The reagent is based on dihydroethidium (DHE), which is a cell-permeable fluorescent dye. Upon entering the cells, DHE and superoxide interact to form red fluorescent oxyethidium. The Muse® Cell Analyzer instrument uses microcapillary cytometry for single-cell analysis and laser-based fluorescence detection of each cell event. Briefly, THP-1 cells were seeded at a density of 5×10^5 cells/ml in 12-well plates and differentiated into macrophages by stimulation with 10 ng/ml PMA. Cells were exposed to 500 µM H_2O_2 or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts for 1 h. The mitochondria-targeted antioxidant mitoquinone mesylate (MitoQ, 2 µg/ml) was used with AGE-BSA as the positive control. MitoQ was chosen due to its protective effect against oxidative damage and inflammatory responses by inhibiting the RAGE signaling pathway [37,38,39,40,41,42,43,44]. Thereafter, the cells were incubated with working reagent for 30 min and were analyzed using the Muse® Cell Analyzer (Merck, Darmstadt, Germany).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

THP-1 cells were seeded in 6-well plates at a density of 1×10^6 cells/ml and differentiated into macrophages by treatment with 10 ng/ml PMA. Then, the cells were exposed to 1 µg/ml LPS or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts or 2 µg/ml MitoQ for 24 h. Total RNA was extracted using TRIzol reagent (Invitrogen, MA, USA) following the manufacturer's instructions. The RNA template was used for cDNA synthesis using the AccuPower® CycleScript RT PreMix Reverse Transcription System (Bioneer, Daejeon, Korea) and oligo(dT)18 primer. For the amplification reaction, qPCR was performed using the Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Daejeon, Korea). mRNA expression was analyzed using the SYBR green primer pairs listed in Table 1. The specificity of the reaction products was assessed by performing melting curve analysis. The expression of each gene was normalized to the housekeeping gene β-actin. The fold change in expression was determined using the ΔΔCt method (2-ΔΔCt).

Western blotting analysis

THP-1 cells were seeded at a density of 1×10^6 cells/ml in 6-well plates and differentiated into macrophages by treatment with 10 ng/ml PMA. The cells were exposed to 1 µg/ml LPS or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts or 2 µg/ml MitoQ for 1 h or 24 h. Proteins were isolated from THP-1 cells using lysis buffer with phosphatase inhibitor. The

protein concentration was measured by the Bradford protein assay (Bio-Rad, CA, USA). Proteins (15 μg) were separated by 10% SDS-PAGE and transferred to PVDF membranes. Unspecific protein-binding sites were blocked by incubating the membrane with TBS-T containing 5% nonfat dry milk for 1 h. The membranes were then incubated with primary antibodies (Table 2) overnight at 4 °C, followed by secondary antibodies (peroxidase-conjugated goat anti-mouse or anti-rabbit IgG, Cell Signaling Technology, MA, USA). The blots were incubated in ECL Select Western blotting Detection Reagent (GE Healthcare, IL, USA) and then visualized using high-performance chemiluminescence (GE Healthcare, IL, USA). The intensities of the protein bands were quantitated using ImageJ software (National Institute of Health, MD, USA), and β-actin was used as the housekeeping protein.

Statistical analysis

The results of 3 repeats are presented as the mean ± standard error of the mean (SEM) and were analyzed using one-way ANOVA with post hoc Bonferroni tests (Prism 7, GraphPad, CA, USA). A p value < 0.05 was considered statistically significant.

Results

Antioxidant capacity of *F. ovata* extracts

The results in Table 3 show that the dichloromethane and methanol extracts of *F. ovata* showed a similar stronger antioxidant capacity than petroleum ether extract determined by both the ABTS and the DPPH assay ($P < 0.05$).

Antioxidant capacity of *A. vulgaris* extracts

The results in Table 4 show that the antioxidant capacities of the dichloromethane and methanol extracts of *A. vulgaris* were significantly higher than that of the petroleum ether extract, as determined by the ABTS assay ($p < 0.05$). In the DPPH assay, the methanol extract of *A. vulgaris* showed the highest antioxidant capacity ($p < 0.05$ vs. dichloromethane and petroleum ether extracts), followed by the

dichloromethane extract ($p < 0.05$ vs. petroleum ether), and the petroleum ether extract showed the lowest antioxidant capacity (Table 4).

Effect of *F. ovata* and *A. vulgaris* extracts, AGE-BSA, and BSA on cell viability

Upon incubation with various concentrations (0.78–100 µg/ml) of *F. ovata* and *A. vulgaris* extracts for 24 h, no significant change in the viability of THP-1 cells was observed (Fig. 1 a-b). Upon incubation with various concentrations of AGE-BSA and BSA (25–600 µg/ml) for 24 h, there was no significant change in cell viability (Fig. 1c).

Effect of *F. ovata* and *A. vulgaris* extracts on AGEs-induced intracellular superoxide radical production

Treatment with H_2O_2 and AGE-BSA for 1 hour increased intracellular superoxide radical production by 30% (Fig. 2). Treatment with 100 µg/ml petroleum ether, dichloromethane and methanol extracts of *A. vulgaris* marginally reduced superoxide radical production when compared with AGE-BSA treatment group. The positive control MitoQ at the concentration of 2 µg/ml did not affect the cell viability of THP-1 cells (Supplementary Fig S1). Treatment with MitoQ normalized superoxide radical production to the Untreated level (Fig. 2).

Effect of *F. ovata* and *A. vulgaris* extracts on AGEs-induced RAGE expression

Compared with the control condition, LPS treatment for 24 hours increased RAGE protein expression by 50% without statistical significance (Fig 3) and the treatment with AGE-BSA for 24 hours significantly increased RAGE protein expression ($P < 0.05$ vs Untreated; Fig. 3). In addition, the treatment with the dichloromethane extract of *F. ovata* and *A. vulgaris* and methanol extract of *A. vulgaris* significantly attenuated RAGE protein expression when compared with the AGE-BSA group ($P < 0.05$; Fig. 3). However, petroleum ether extract of *F. ovata* and *A. vulgaris* and methanol extract of *F. ovata* did not affect RAGE protein levels. The treatment with MitoQ normalized RAGE protein

expression when compared with AGE-BSA group ($P < 0.05$; Fig. 3).

Effect of *F. ovata* and *A. vulgaris* extracts on MAPKs signaling

Upon treatment with LPS and AGE-BSA for 1 h, the levels of phospho-Erk1/2 (Fig. 4a), total Erk1/2 (Fig. 4b), and phospho-JNK (Fig. 5a) were increased by 50%, although without statistical significance. The total JNK levels were increased marginally by LPS treatment and by 50% in the AGE-BSA treatment (Fig. 5b). Compared with the control condition, both the LPS and AGE-BSA treatments increased phospho-p38 MAPK levels by 70% but only marginally increased p38 MAPK levels without statistical significance (Fig. 6a, b). There was no change in the Erk1/2, JNK, and p38 MAPK protein levels between the AGE-BSA group and the plant extract groups (Figs. 4, 5, 6). MitoQ treatment marginally reduced phosphorylated and total Erk1/2, JNK, and p38 MAPK levels, although without statistical significance when compared with AGE-BSA treatment (Figs. 4, 5, 6). There was no change in phospho-Erk1/2/total Erk1/2, phospho-JNK/total JNK, or phospho-p38 MAPK/total p38 MAPK between all groups.

Effect of *F. ovata* and *A. vulgaris* extracts on NF-κB expression

The protein levels of the transcription factor NF-κB were increased by 40% and 60% by LPS and AGE-BSA treatments, respectively, compared with the control condition (Fig. 7). There was no difference in NF-κB levels between the AGE-BSA group and the plant extract groups, whereas MitoQ normalized NF-κB to the control level.

Effect of *F. ovata* and *A. vulgaris* extracts on AGEs-induced inflammatory cytokines expression

Our data revealed that, LPS and AGE-BSA significantly induced TNF-α, IL-1β, and IL-6 mRNA expression ($P < 0.05$ vs Untreated; Fig. 8a-c). Treatment with all extracts of *F. ovata* and *A. vulgaris* normalized TNF-α mRNA expression ($P < 0.05$ vs AGE-BSA; Fig. 8a). IL-1β mRNA expression was significantly decreased ($P < 0.05$ vs AGE-BSA; Fig. 8b) when treated with the methanol extract of *F. ovata* and all extracts of *A. vulgaris*. Moreover, all the extracts of *F. ovata* and petroleum ether extract

of *A. vulgaris* normalized IL-6 mRNA expression ($P < 0.05$ vs AGE-BSA; Fig. 8c). MitoQ significantly inhibited TNF-α, IL-1β, and IL-6 mRNA expression when compared with the AGE-BSA group ($P < 0.05$; Fig. 8a-c).

Discussion

In this study, we assessed the antioxidant and anti-inflammatory capacity of *F. ovata* and *A. vulgaris* extracts. Different methods of extraction were examined. Results from our study suggested that the dichloromethane and methanol extracts of *F. ovata* had the same high antioxidant activity, while the methanol extract of *A. vulgaris* had the highest antioxidant activity. All extracts of *F. ovata* and *A. vulgaris* showed potent anti-inflammatory effects, which was associated with the inhibition of AGE induced RAGE expression.

The radical-scavenging capacity of plant extracts was dependent on solvent polarity, which is related to the polar nature of the active compounds in each plant. Among the major classes of phytochemicals, phenolic compounds are the most extensively studied, especially focusing on health benefits due to its potential against oxidative damage (39). Polar solvents are efficiently used for recovering phenolic compounds from plants (40). Our results indicated that the natural antioxidants in *F. ovata* and *A. vulgaris* may mainly be preserved in the polar solvent extracts. It is noticeable that there are some differences in the radical-scavenging capacity of plant extracts detected by DPPH and ABTS assay. The ABTS assay was superior to DPPH assay and more sensitive in identifying the antioxidant activity since it has faster reaction kinetics. It is also useful for assessing the antioxidant capacity of samples extracted in acidic solvents and samples containing hydrophilic, lipophilic, and pigment compounds (41, 42). However, none of the *F. ovata* and *A. vulgaris* extracts have shown marked antioxidant activity in cells, whereas ROS production induced by AGE was inhibited by the known mitochondrial targeting antioxidant MitoQ. This may be due to the dose of *F. ovata* and *A. vulgaris* extracts used in this study.

AGEs-RAGE interaction leads to an increase in oxidative stress, and activation of various cell

signaling pathways including MAPKs, PI3K-Akt, and NF-κB, which causes the expression of a variety of inflammation-related genes and promotes inflammation (7, 8). There is increasing evidence suggesting AGEs-RAGE axis as the therapeutic target for chronic inflammation-related conditions. AGEs inhibitor could prevent oxidative stress and have protective effects against inflammation (43). In addition, clinical research revealed that sRAGE acting as a RAGE competitor could suppress vascular inflammation (44). Moreover, the knockout of RAGE gene resulted in reduced atherosclerosis and vascular inflammation (45). Therefore, targeting RAGE could be a therapeutic strategy for the treatment of conditions due to oxidative stress and chronic inflammation mediated by AGEs-RAGE axis.

In this study, AGEs induced RAGE expression and RAGE downstream signaling molecules including Erk1/2, JNK, p38 MAPK, and NF-κB, as well as inflammation-related genes including TNF-α, IL-1β, and IL-6. Most *F. ovata* and *A. vulgaris* extracts exerted a suppressive effect on RAGE expression and inflammatory cytokines production, except for the ether extracts. This anti-inflammatory effect is independent of their antioxidant capacity, at least at the trialed dose. The role of *F. ovata* and *A. vulgaris* extracts on the inhibition of the AGEs are, at least, mediated by attenuating RAGE increase, however without any effect on Erk1/2, JNK, p38 MAPK, and NF-κB. This suggests that another pathway is involved. In addition to MAPKs and NF-κB, among the known signaling cascades that can be activated by RAGE are phosphoinositide 3-kinases (PI3K)/Akt, Protein kinase C, and JAK/STAT pathway (46-48). Previous work showed that both IL-6 and TNF-α contributed to the activity of PI3K/Akt pathway through multiple mechanisms (46, 47). The cytosol-to-membrane translocation of PKC isoforms with the subsequent activation of MAP kinases and AP-1 transcription factor, may represent critical steps in the induction of signaling cascade leading to TNF-α, IL-1β, and IL-6 synthesis in human monocytes (48). Additional studies are needed to discover the alternative pathway mediating the anti-inflammatory effects of *F. ovata* and *A. vulgaris* extracts.

In summary, *F. ovata* and *A. vulgaris* extracts have an inhibitory effect on AGE-mediated RAGE overexpression and inflammatory responses and showed antioxidative effects in non-cell-based assays. Therefore, *F. ovata* and *A. vulgaris* might be useful as alternative options to prevent AGE-RAGE

signaling-mediated inflammatory conditions. Future studies are needed to elucidate the active compounds in various extracts and to confirm the effect of *F. ovata* and *A. vulgaris* extracts on more AGE-RAGE cascade components.

Abbreviations

AGEs: Advanced glycation end products (AGEs); RAGE: receptor for AGEs; ROS: Reactive oxygen species, MAPKs: mitogen-activated protein kinases; ERK1/2: Extracellular signal-regulated protein kinases 1 and 2; JNKs: c-Jun N-terminal kinases; PI3K: phosphoinositide 3-kinase; NF-κB: nuclear factor kappa B; TNF-α: Tumor Necrosis Factor alpha; IL-1β: Interleukin1 beta; IL-6: Interleukin6

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Table 1 Human Syber Green primers for qRT-PCR

Gene	Sequence	Annealing Temperature (°C)	Product Size (bp)
TNF-α	Forward primer 5′ TCTCGAACCCCGAGTGACAA 3′ Reverse primer 5′ TGAAGAGGACCTGGGAGTAG 3′	55	181
IL-1β	Forward primer 5′ ACCAAACCTCTTCGAGGCAC 3′ Reverse primer 5′ CATGGCCACAACAACTGACG 3′	56	300
IL-6	Forward primer 5′ GAAGAGAGCCCTCAGGCTGGACTG 3′ Reverse primer 5′ TGAACTCCTTCTCCACAAGCGC 3′	64	627
RAGE	Forward Primer 5’ GTGGGGACATGTGTGTCAGAGGGAA 3’ Reverse Primer 5’ TGAGGAGAGGGCTGGGCAGGGACT 3’	64	383
β-actin	Forward Primer 5’ ACGGGTCACCCACACTGTGC 3’ Reverse Primer 5’ CTAGAAGCATTTGCGGTGGACGATG 3’	58	656

Table 2 List of antibodies for Western blotting analysis

Primary antibody	dilution factor	Manufacture
phospho-Erk1/2	1:4,000	Cell Signalling Technology, MA, USA

Erk1/2	1:4,000	Cell Signalling Technology, MA, USA
phospho-JNK	1:2,000	Cell Signalling Technology, MA, USA
JNK	1:2,000	Cell Signalling Technology, MA, USA
phospho-p38 MAPK	1:4,000	Cell Signalling Technology, MA, USA
p38 MAPK	1:4,000	Cell Signalling Technology, MA, USA
NF-κB	1:2,000	Cell Signalling Technology, MA, USA
RAGE	1:1,000	Merck, Darmstadt, Germany
β-actin	1:10,000	Cell Signalling Technology, MA, USA

Table 3 Antioxidant capacity of *F. ovata* extracts determined by ABTS and DPPH assays.

F. ovata. **Extracts**	**ABTS (mg VCEAC/g)**	**DPPH (mg VCEAC/g)**
Petroleum ether	10.8±0.4	11.87±0.2
Dichloromethane	67.5±2.6*	47.8±2.8*
Methanol	62.9±1.0*	47.1±1.2*

Data are presented as Mean ± SE, n = 3. *$P < 0.05$ vs petroleum ether extract.

Table 4 Antioxidant capacity of *A. vulgaris* extracts determined by ABTS and DPPH assays.

A. vulgaris. **Extracts**	**ABTS (mg VCEAC/g)**	**DPPH (mg VCEAC/g)**
Petroleum Ether	18.0±3.6	15.4±0.1
Dichloromethane	90.7±0.5*	40.5±1.1*
Methanol	99.2±0.2*	88.4±0.1*#

Data are presented as Mean ± SE, n=3. $^{*}P < 0.05$ vs petroleum ether extract, $^{\#}P < 0.05$ vs dichloromethane extract.

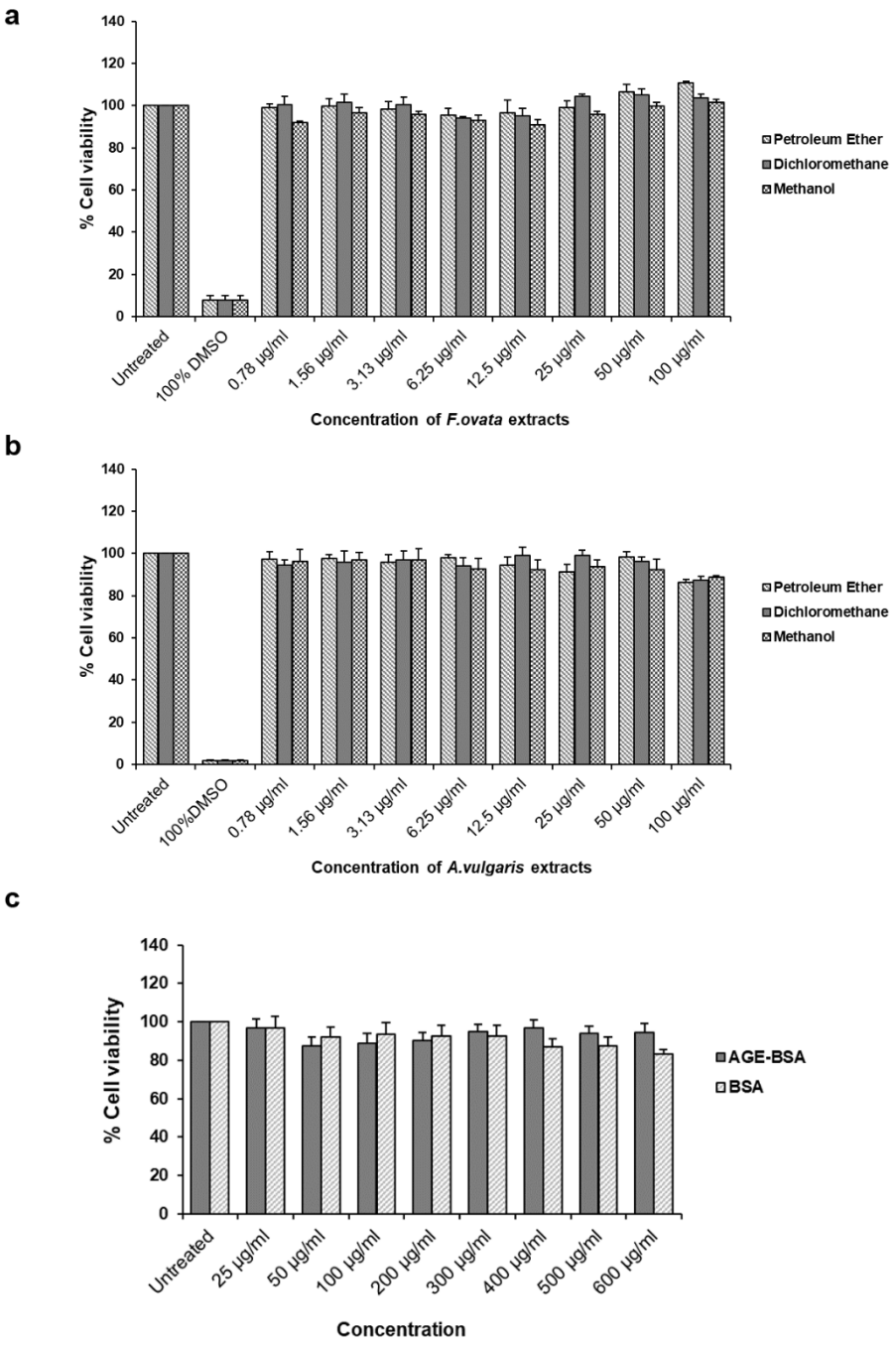


Fig. 1 Cell viability of THP-1 cells. Cells were exposed to *F.ovata* (a) and *A.vulgaris* (b) extracts, AGE-BSA and BSA (c) for 24 hours. Cell viability was determined by the MTS assay. Results are expressed as means ± SE, n = 3.

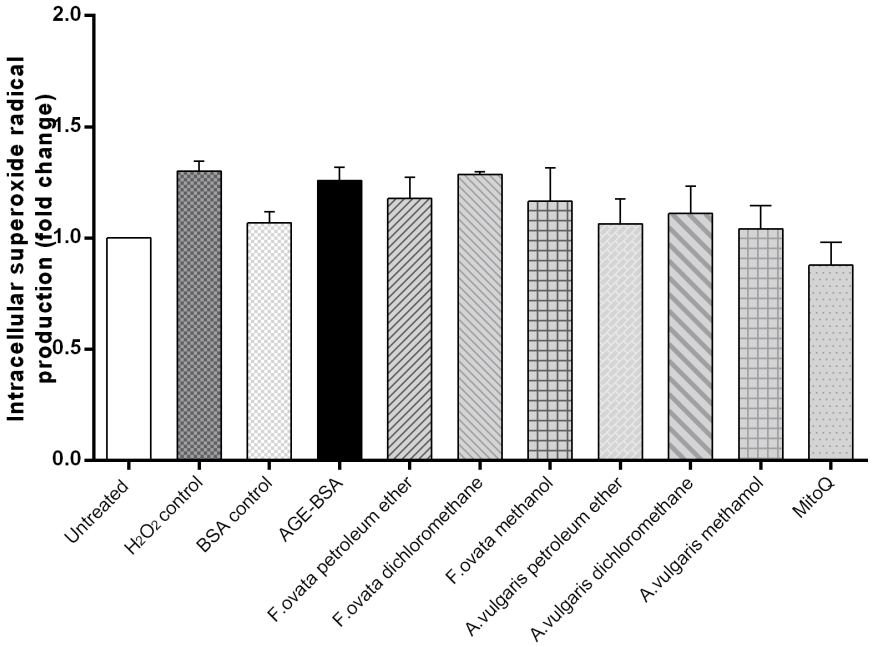


Fig. 2 Intracellular superoxide radical production in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts for 1 hour. H_2O_2 was used as a negative control and MitoQ was used as a positive control. Results are expressed as means ± SE, n=3.

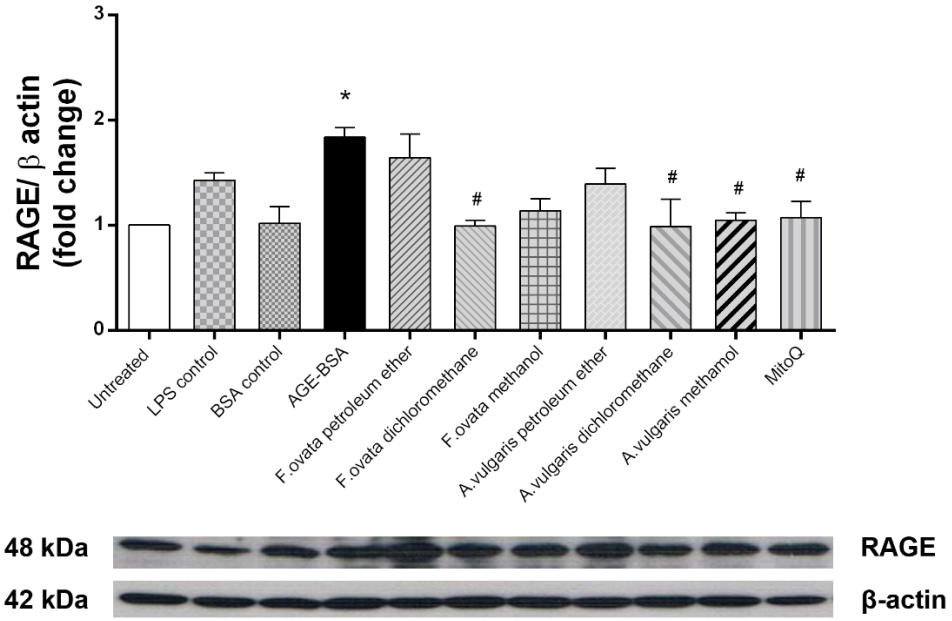


Fig. 3 Protein expression of receptors for advanced glycation end products (RAGE) in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts or MitoQ for 24 hours. MitoQ was used as a positive control. Results are expressed as means ± SE, n = 3. $^{*}P < 0.05$ AGE-BSA vs Untreated, $^{\#}P < 0.05$, MitoQ and plant extracts vs AGE-BSA.

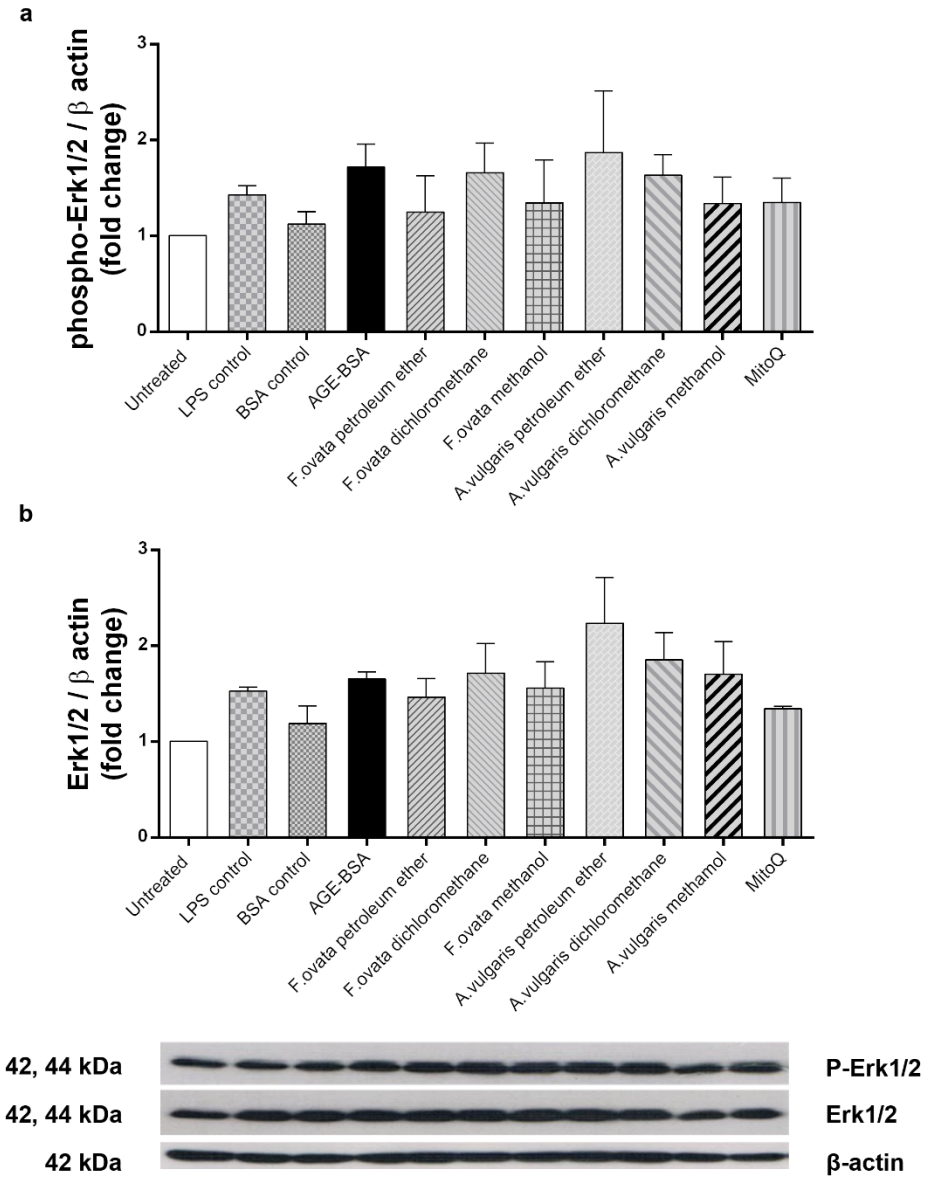


Fig. 4 Phospho-Erk1/2 (a) and total Erk1/2 (b) protein levels in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts or MitoQ for 1 hour. Results are expressed as means ± SE, n = 3. $^{*}P < 0.05$ AGE-BSA vs Untreated.

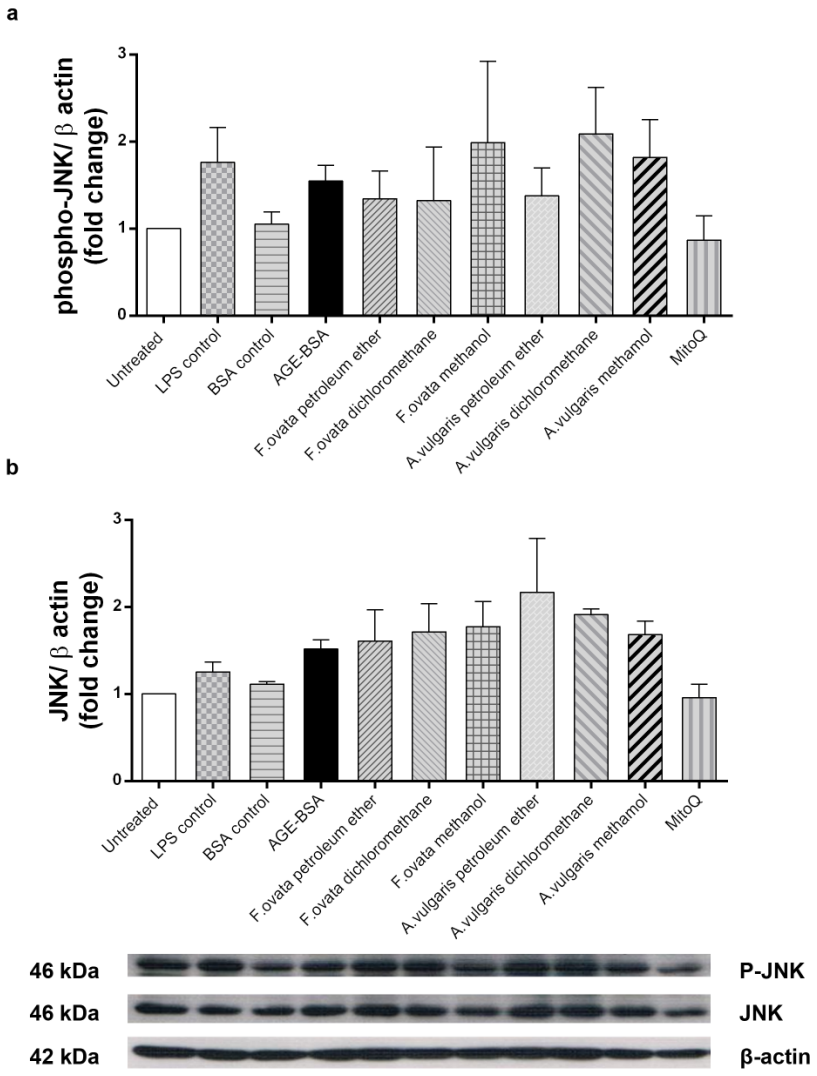


Fig. 5 Phospho-JNK (a) and total JNK (b) protein levels in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts or MitoQ for 1 hour. Results are expressed as means ± SE, n = 3. $^{*}P < 0.05$ AGE-BSA vs Untreated.

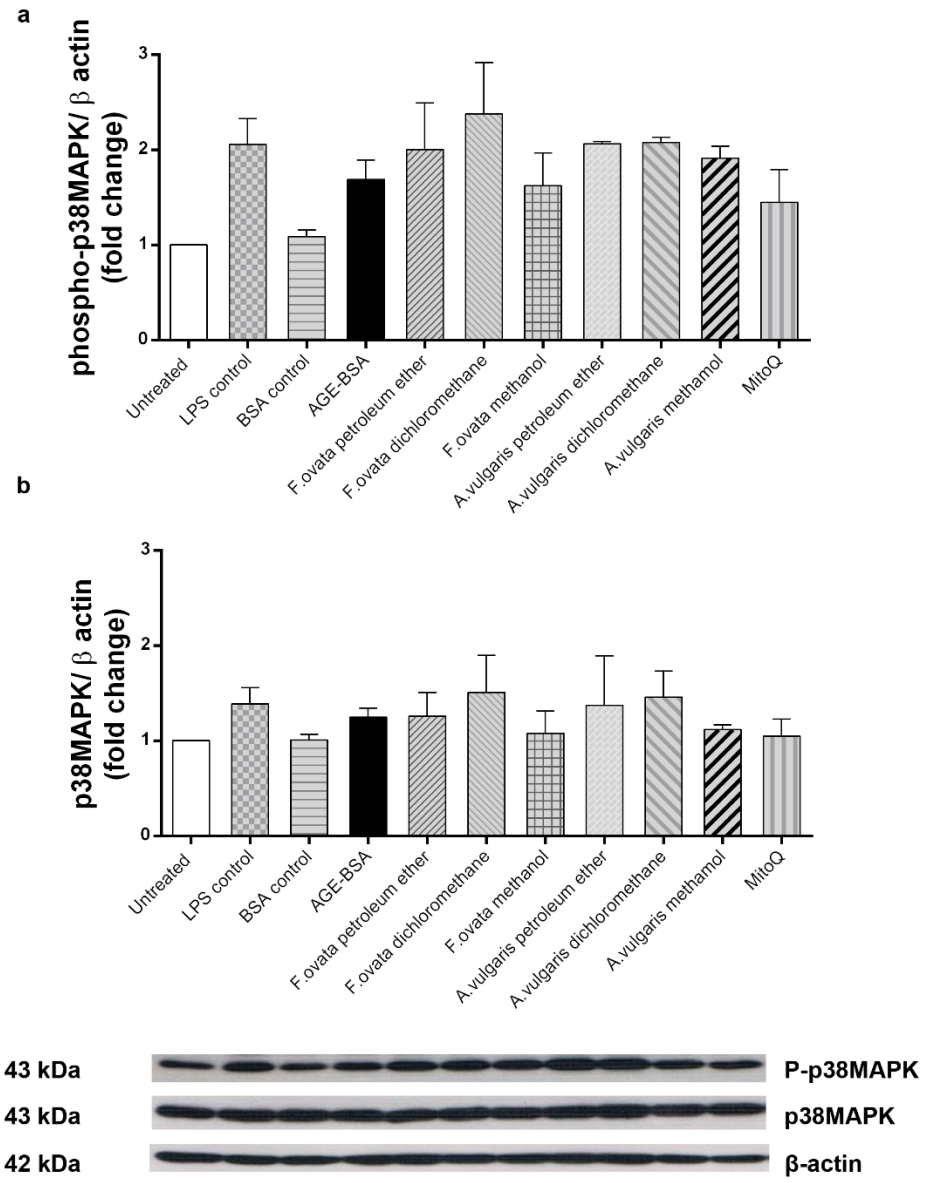


Fig. 6 Phospho-p38MAPK (a) and total p38MAPK (b) protein levels in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts for 1 hour. MitoQ was used as a positive control. Results are expressed as means ± SE, n = 3. $^{*}P < 0.05$ AGE-BSA vs Untreated.

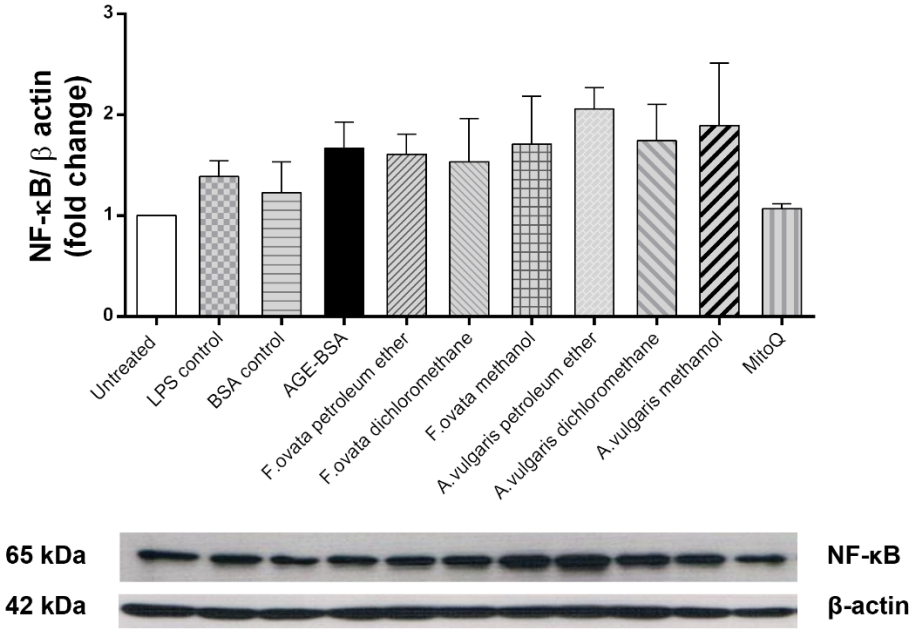


Fig. 7 NF-κB protein levels in THP-1 cells. Cells were exposed to AGEs-BSA alone or AGEs-BSA in combination with plant extracts for 1 hour. MitoQ was used as a positive control. Results are expressed as means ± SE, n = 3.

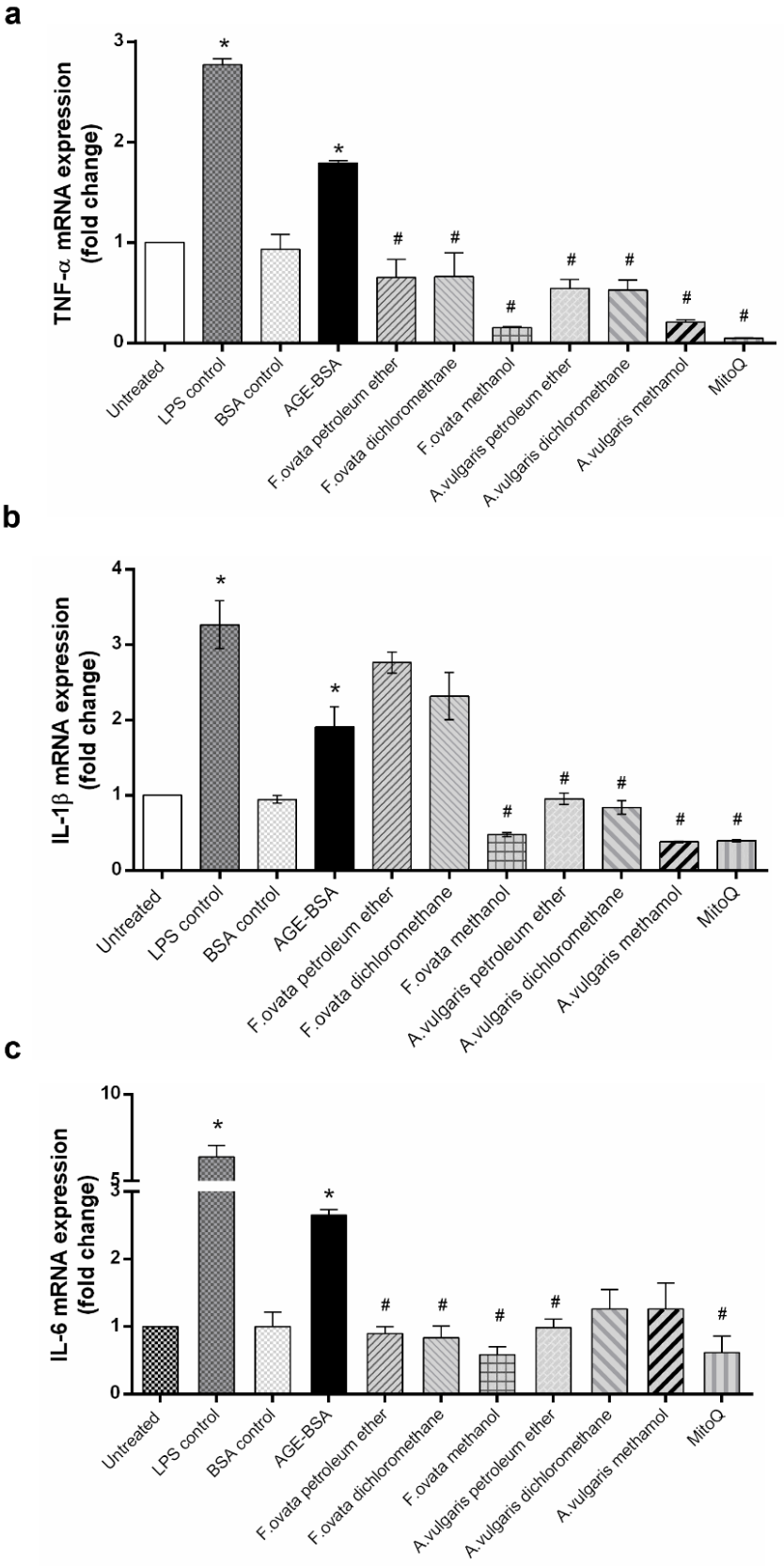
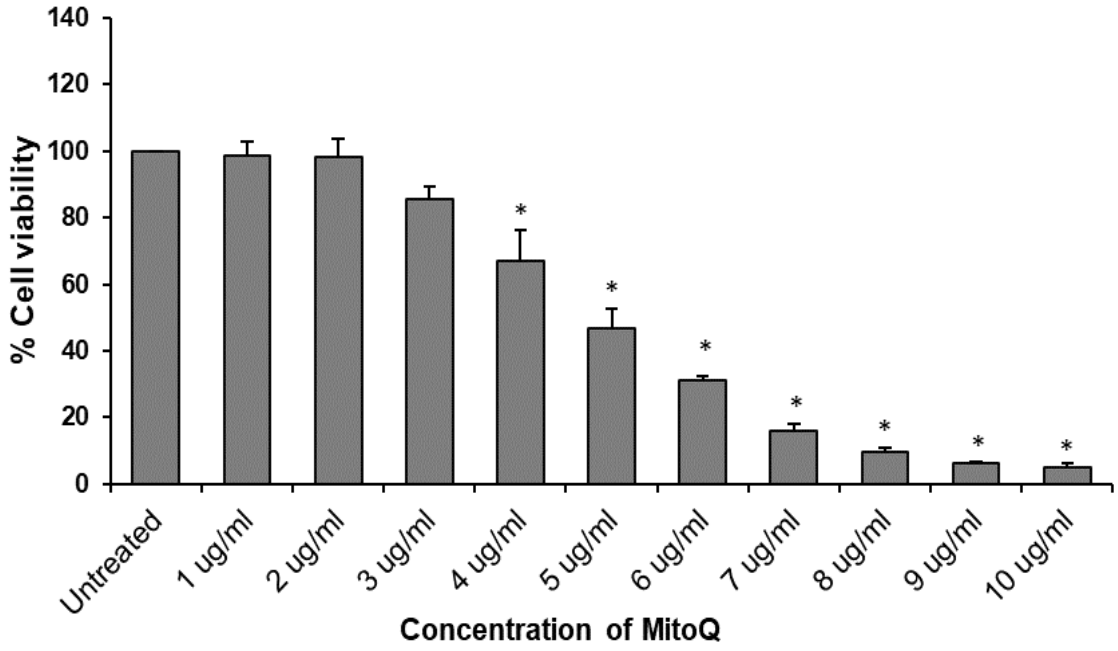


Fig. 8 TNF-α (a), IL-1β (b), and IL-6 (c) mRNA expression in THP-1 cells. Cells were exposed to AGEs-

BSA alone or AGEs-BSA in combination with plant extracts or MitoQ for 24 hours. Results are expressed as means ± SE, n = 3. $^{*}P< 0.05$ AGE-BSA vs Untreated, $^{\#}P< 0.05$ MitoQ and plant extracts vs AGE-BSA.



Supplementary Fig. S1 Cell viability of THP-1 cells. Cells were exposed to MitoQ for 24 hours and then cell viability was determined by the MTS assay. Results are expressed as means ± SE, n = 3. $^{*}P < 0.05$ vs Untreated.