Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2, 340-354
2026
Publisher: Learning Gate
DOI: 10.55214/2576-8484.v10i2.12061

Assessment of anti-inflammatory effects of Micromeria barbata ethanolic extract and AKBA in AGE-induced inflammation in PBMC and THP-1 cell lines

May Saad[1], Abeer J. Ayoub[2,3,4], Mahmoud I. Khalil[1,5*], Nadine Nasreddine[6,7]
[1]Department of Biological Sciences, Faculty of Science, Beirut Arab University, Beirut, Lebanon; maysaad551@gmail.com (M.S.) m.khalil@bau.edu.lb (M.I.K.).
[2]Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Lebanon; abeer.ayoub@liu.edu.lb (A.J.A.).
[3]Laboratory of Cancer Biology and Molecular Immunology, Faculty of Science I, Lebanese University, Hadath 1104, Lebanon.
[4]Department of Biological Sciences, School of Arts and Sciences, Lebanese International University, Bekaa Campus, Bekaa, Lebanon.
[5]Molecular Biology Unit, Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.
[6]Cancer and Molecular Biology Lab, Faculty of Science, Lebanese University, Beirut, Lebanon; nadineelghotmi@ul.edu.lb (N.N.).
[7]Department of Microbiology, Faculty of Public Health, Lebanese University, Saida, Lebanon.

Abstract: This study investigated the anti-inflammatory effects of Micromeria Barbata (MB) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) in human PBMCs and THP-1 cells following stimulation with advanced glycation end products (AGEs). Cytotoxicity of MB, AKBA, and AGE-BSA was evaluated using MTT assays. Subsequently, RT-PCR was employed to quantify cytokine responses and assess how MB and AKBA modulated IL-6, TNF-α, and IL-1β expression in PBMCs and THP-1 cells. MTT assays confirmed that MB (5–40 µg/mL), AKBA (0.01–0.5 µg/mL), and AGE-BSA (10–100 µg/mL) were non-toxic under 24–48 hours exposure. AGE-BSA (10 µg/mL) significantly increased cytokine expression in PBMCs (IL-6, IL-1β, TNF-α: 115-, 11.7-, 0.7-fold) and THP-1 cells (325-, 11-, 7.3-fold). Pretreatment with MB or AKBA mitigated this inflammatory response in a dose-dependent manner. MB (40 µg/mL) reduced IL-6, IL-1β, and TNF-α to 0.2, 0.19, and 0.3-fold, whereas AKBA (0.3 µg/mL) resulted in 0.7, 0.6, and 0.46-fold. The study revealed that both MB and AKBA pretreatment reduced inflammatory responses in a dose-response manner to AGE-BSA in PBMC and THP-1 cells. This study offers new insights into treating AGE-induced complications and highlights potential in treating diabetes.

Keywords: *Acetyl-11-keto-β-boswellic Acid (AKBA), Advanced glycation end products (AGEs), Cell-specific responses, Inflammation, Micromeria barbata (MB).*

1. Introduction

Advanced glycation end products (AGEs) are formed when a reducing sugar reacts non-enzymatically with a lipid, nucleic acid, or free amino group in a protein. This reaction initiates molecular changes, yielding an irreversible complex of glycated products [1]. AGEs, whether exogenous or endogenous, play key roles in developing various diseases, including atherosclerosis, cardiovascular disease, and diabetes [1]. Under diabetic conditions, chronic hyperglycemia enhances the rate of non-enzymatic glycation of plasma proteins by glucose, leading to protein structural modification and loss of biological activity [2].

Peripheral blood mononuclear cells (PBMCs) are essential components in an individual's innate and adaptive immunity [3, 4]. They recognize specific antigens and activate T and B lymphocytes.

History: Received: 2 December 2025; Revised: 20 January 2026; Accepted: 23 January 2026; Published: 6 February 2026
* Correspondence: m.khalil@bau.edu.lb

Additionally,they actively combat infections, identify and remove various molecular and cellular threats, control the cellular microenvironment, and alter the metabolism of cells [5]. The PBMCs population is composed of lymphocytes (NK, T, and B cells), dendritic cells, and monocytes. The relative composition of PBMCs varies across individuals [6, 7]. Monocytes and macrophages are cells of the immune system that recognize and engulf bacteria, fungi, and viruses via signaling interactions mediated by pattern recognition receptors (PRR) and components of the immune system, such as pathogen-associated molecular patterns (PAMPs) [8]. They promote the proliferation of immune cells that eliminate and clear pathogens, and they also release pro-inflammatory chemokines and cytokines [9, 10]. THP-1 is a human monocytic cell line widely used as a model in mechanistic studies to investigate cellular attributes, signaling pathways, and immune systems due to its ability to differentiate into macrophages [11]. In PBMCs, AGEs are important modulators of immune activity.

Certain studies indicate that AGEs can trigger an immune response, mainly by stimulating pathways such as lymphocyte proliferation, monocyte expression of ICAM-1 and CD40, and secretion of tumor necrosis factor-alpha (TNF-alpha) [12]. Such immune system dysfunction is mainly due to the interaction of AGEs and their appropriate specific receptors, RAGE [13]. RAGE localizes not only on monocytes, but also on T lymphocytes. RAGE engagement on T cells is reported to enhance immune response by stimulating antigen-specific T cell proliferation [14]. RAGE signaling involving cytokines also explains the finding, underscoring its potential as an anti-inflammatory intervention [15]. AGEs were also found to hyperactivate the monocytic cell line THP-1, an aspect of the innate immune system [16]. For these reasons, AGEs are reported to induce the secretion of TNF-α and IL-6 and to promote the differentiation of THP-1 cells into macrophages.

Due to the recently discovered possible applications of herbal remedies with anti-inflammatory effects, the field of herbal medicine is receiving renewed attention as a viable alternative treatment for managing symptoms associated with inflammatory conditions. *Micromeria barbata* (MB) is a flowering plant known for producing oils with diverse chemical compositions; *Micromeria*, the broader genus of MB, has 78 species and is a member of the mint family. The binding of AGE to RAGE triggers a cascade of redox-activated keratin factors, notably NF-κB and STAT3, which drive the synthesis of pro-inflammatory factors (TNF-α, IL-6, and IL-1β) [17-19]. MB is rich in several polyphenolic and terpenoid antioxidants [20] that can scavenge reactive oxygen species (ROS) [21]. Thus, it is plausible to argue that MB disrupts the AGE-RAGE signaling pathway by lowering oxidative stress, which causes a reduction in the expression of NF-κB translocation to the nucleus. The effects are expected to down-regulate pro-inflammatory cytokine gene expression as well as imbalanced ROS. Despite recent reports highlighting the pharmacological properties of MB essential oil, such as its antimicrobial and antifungal activities [22, 23] antioxidant, anticancer, and antidepressant effects [24] no studies, to the best of our knowledge, have specifically investigated the anti-inflammatory effects of MB extract in relation to AGEs and diabetes. This gap prompts the current study of MB ethanolic extract on AGE-BSA–induced cytokine responses in PBMC and THP-1 cells.

Boswellia serrata is a medicinal plant known for its analgesic [25] anti-proliferative [26] and anti-arthritis properties [27] due to its potency in mitigating reduced oxidative stress and inhibiting NF-κB, AKBA has been shown to inhibit NF-κB, prevent IκBα phosphorylation, and modulate MAPK signaling, thus blocking the transcription of pro-inflammatory mediators [28, 29]. Also, through NF-κB inhibition, AKBA suppresses T-lymphocyte proliferation and activation, preserving T-lymphocytes without inducing cytotoxicity. AKBA influences microglia polarization in injured spinal cords and suppresses inflammatory responses by reducing adhesion molecules and other inflammatory mediators [30]. Although the anti-inflammatory effect is well investigated, AKBA effects remain unverified in the context of AGE-induced inflammation, particularly in studies involving PBMC and THP-1 cell lines. AKBA may function as a downstream inhibitor of RAGE-mediated NF-κB signaling, thereby restricting cytokine release and immune activation.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

In this research, we hypothesize that MB ethanolic extract and AKBA will suppress AGE-BSA–induced pro-inflammatory cytokine expression in immune cells by inhibiting the RAGE/NF-κB signaling pathway. The objective is to achieve a deeper understanding of the fundamental mechanisms and, consequently, the therapeutic potential of MB and AKBA in influencing inflammatory responses, especially concerning AGE-related pathogenicity, such as diabetes.

2. Materials and Methods

2.1. Reagents

3-Acetyl-11-keto-β-boswellic acid (AKBA), fetal bovine serum (FBS), MTT reagent, dimethylsulfoxide (DMSO), human interleukin (IL-2), phosphate-buffered saline (PBS), RPMI 1640 with L-glutamine, penicillin-streptomycin, TRIzol, lipopolysaccharide (LPS), phorbol myristate acetate (PMA #P1585), Ficoll-Hypaque, and phytohemagglutinin (PHA) were purchased from Sigma-Aldrich (St. Louis, USA). AGE-BSA was sourced from Abcam Ltd. (Cambridge, UK) (ab51995). Bio-Rad (Hercules, California) supplied the iScript synthesis kit for reverse transcription along with the iTaq universal SYBR Green supermix for PCR. The human monocytic cell line THP-1 and primers were kindly provided by the American University of Beirut from Dr. Nadine Darwish's Lab, Faculty of Medicine, Biochemistry Department. The MB plant extract was generously provided by the Lebanese University's Doctoral School of Sciences and Technologies at the Azm Center in Tripoli. Whole blood (WB), freshly drawn from healthy volunteers, was generously provided by the Military Hospital, Beirut, Lebanon, after gaining verbal consent from the patients and written consent from the hospital.

2.2. Preparation of Plant Extract

Sections of the leaves, flowers, and stem of MB were collected and dried at room temperature, and the samples were finely ground. The chemical characterization of the MB extract was not performed in the current study. However, a recent investigation by Rawas et al. [22] analyzed the phytochemical constituents of MB using FTIR and GC-MS. The study revealed a rich composition of various secondary metabolites, including verbenone, piperitenone, oleic acid, and other identified components. For extract preparation, at room temperature, 40 g of plant powder was soaked in 70% ethanol for 48 hours. The subsequent phase was decanted, followed by filtration through Whatman N°1filter paper. A rotating evaporator was used to remove the ethanol from the extract at a temperature of 60°C and low pressure. The eluates obtained after evaporating the ethanol were processed using lyophilization, while the dried samples were stored in sealed dark containers to protect them from light and moisture, which could affect their stability. A 0.5 milligram per milliliter (mg/ml) stock solution of the MB plant extract was prepared in DMSO.

2.3. PBMC Isolation and THP-1 Culturing

Collection of peripheral blood was done in EDTA tubes from a healthy adult male donor (aged 30-40 years old), and PBMCs were separated and purified using Ficoll-Hypaque. According to the manufacturer's instructions, the blood was centrifuged for thirty minutes at room temperature and 400 g to create a density gradient. Briefly, 60 mL of freshly drawn blood was mixed at a ratio of 1:1 (vol/vol) with PBS 1X at room temperature, and 30 mL of the mixture was layered on 15 mL of Ficoll-Paque PLUS in 50 mL Falcon tubes. Then, it was centrifuged at 1800 rpm for 30 minutes at room temperature without a break. The PBMC layer was collected, washed with PBS 1X, and centrifuged at 1200 rpm for 10 minutes at room temperature without a break to remove the remaining Ficoll solution. PBMCs were collected in a cell culture medium. Cell number and viability were determined using a hemocytometer and trypan blue. The PBMCs were suspended with RPMI 1640, 1% FBS, 1% penicillin/streptomycin, and 10 ng/mL human IL-2 for subsequent experiments. For the MTT assay, PBMC cells were seeded in 96-well flat-bottom cell culture plates at a density of 5×10^4 cells per well. They were then subjected to AKBA (0.01, 0.03, 0.125, and 0.5 µg/mL), AGE-BSA (10, 25, 50, and 100

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

μg/mL), and MB-ethanolic extract (5, 10, 20, and 40 μg/mL). Cells were also cultivated in a mixture of AKBA and MB at different doses (0.01, 0.03, 0.125, and 0.5 μg/mL for AKBA and 5, 10, 20, and 40 μg/mL for MB). At 37°C, 5% CO_2, and 95% humidity, the incubation duration was increased to 24 and 48 hours. For RT-PCR, PBMC cells were adjusted to a density of 1×10^6 cells/mL with new culture growth media with 1% fetal bovine serum (FBS) in the presence of 10 ng/mL human IL-2. Then, 1 mL of PBMC cell suspension was inoculated into 12-well cell culture plates. Cells were treated with different agents twenty-four hours after seeding to study their effects on secreted cytokine levels.

THP-1 cells were grown in RPMI 1640 culture medium with L-glutamine supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were seeded in 75 cm^2 flasks for tissue culture until confluent. For the RTPCR experiment, THP-1 cell numbers were counted and adjusted to a density of 1×10^6 cells/mL in fresh culture medium in the presence of 25 nM PMA, and then 1 mL of cell suspension was inoculated into 12-well flat-bottom cell culture plates at 1×10^6 cells per well. Seventy-two hours after seeding, cells were washed with a new culture medium and treated with different agents to study their effects on secreted cytokine levels.

2.4. Cell Viability Assay (MTT)

PBMC cells underwent MTT tests [31] following treatment with AKBA, AGE-BSA, and MB-ethanolic extract to assess cell viability. For the experimental group, cells were grown in full RPMI-1640 as controls. After stimulation, the cells were incubated with 10 μl of MTT reagent (5 mg/mL) for 4 hours at 37°C to assess cell viability. Next, 100 μl of stop solution consisting of 1% DMSO was added to each well to solubilize the formed formazan as an indicator of cell viability, and the cells were incubated for 30 minutes at room temperature in the dark. Absorbance was then measured using an ELISA (enzyme-linked immunosorbent assay) reader at 570 nm to determine the extent of formazan production. Cell viability percentage was calculated using Equation 1: $Percentage\,(\%) = 100\,x\,\frac{Sample\ abs}{Control\ abs}$

2.5. Measurement of Cytokine mRNA by RT-PCR for PBMC and THP-1 Cells

After the incubation time with different agents, the supernatant in each well was kept at -80°C, and the cells were removed using TRIzol Reagent. mRNA expression was assessed by real-time PCR, and the primer sequences used in this study are listed in Table 1. These primers target key inflammatory and oxidative stress genes, enabling precise evaluation of gene expression changes associated with BPA-induced nephrotoxicity.

Table 1.
Primer sequences used in real-time PCR.

Gene	Primers	Reference
IL-1β (NG_008851.1)	F: 5′-TGGCATTGATCTGGTTCATC-3′ R: 5′-GTTTAGGAATCTTCCCACTT-3′	Karakaxas et al. [32]
IL-6 (NG_011640.1)	F: 5′-CACCGAGCTCACCCCACTACC-3′ R: 5′-CTACATTATCCGAACAG-3′	Figueiredo et al. [33]
IL-4 (NG_023252.1)	F: 5′-TGGCCCGAAGAACACAGATG-3′ R: 5′-C TTGAGGTTCCTGTCCAGTCC-3′	Saini et al. [34]
TNF-α (NG_007462.1)	F: 5′-CTGGGGCCTACAGCTTTGAT-3′ R: 5′-GGCTCCGTGTCTCAAGGAAG-3′	Saxena et al. [35]

Total RNA was isolated using TRIzol (Sigma-Aldrich, T9424). The amount and quality of RNA were determined using a Spectrophotometer Nanodrop (Thermo Fisher Scientific) after suspending in 20 μl of RNase- and DNase-free water. Reverse transcription and PCR were designed as a two-step reaction. Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit with 2 μg of total RNA as a template. The reaction was conducted in an RT-PCR machine under the

following conditions: 10 min at 25°C, 2 hours at 37°C, followed by 5 min at 85°C, and ending at 4°C. PCR was carried out using SYBR Green Supermix. The PCR reaction included an initial denaturation step at 94°C for 15 minutes, followed by 50 cycles of denaturation at 94°C for 15 seconds, annealing at 56°C for 9 seconds, and extension at 72°C for 30 seconds using the CFX384 system. The results were quantified and analyzed using the Delta-Delta CT method [36] and normalized to the housekeeping gene GAPDH.

2.6. Statistical Analysis

GraphPad Prism 9 (GraphPad Software, San Diego, CA) was the software used to perform the statistical analysis for this study. All values are expressed as means of triplicate experiments ± SEM. ONE-WAY ANOVA was employed to compare multiple variables. Results with a p-value less than 0.05 were considered statistically significant.

3. Results

3.1. Cytotoxic Effects of AGE, MB, and AKBA in PBMC Cells

PBMC cytotoxicity assessments showed no effects from any AGE, MB, or AKBA concentration at either 24 or 48 hours. For PBMC, AGE-BSA (10, 25, 50, and 100 µg/ml) didn't cause any detected cytotoxic effect at either 24 or 48 hours, and 10 µg/ml of AGE-BSA was used to assess therapeutic effects on cells. MB and AKBA also did not induce detectable cytotoxic effects (Figure 1).

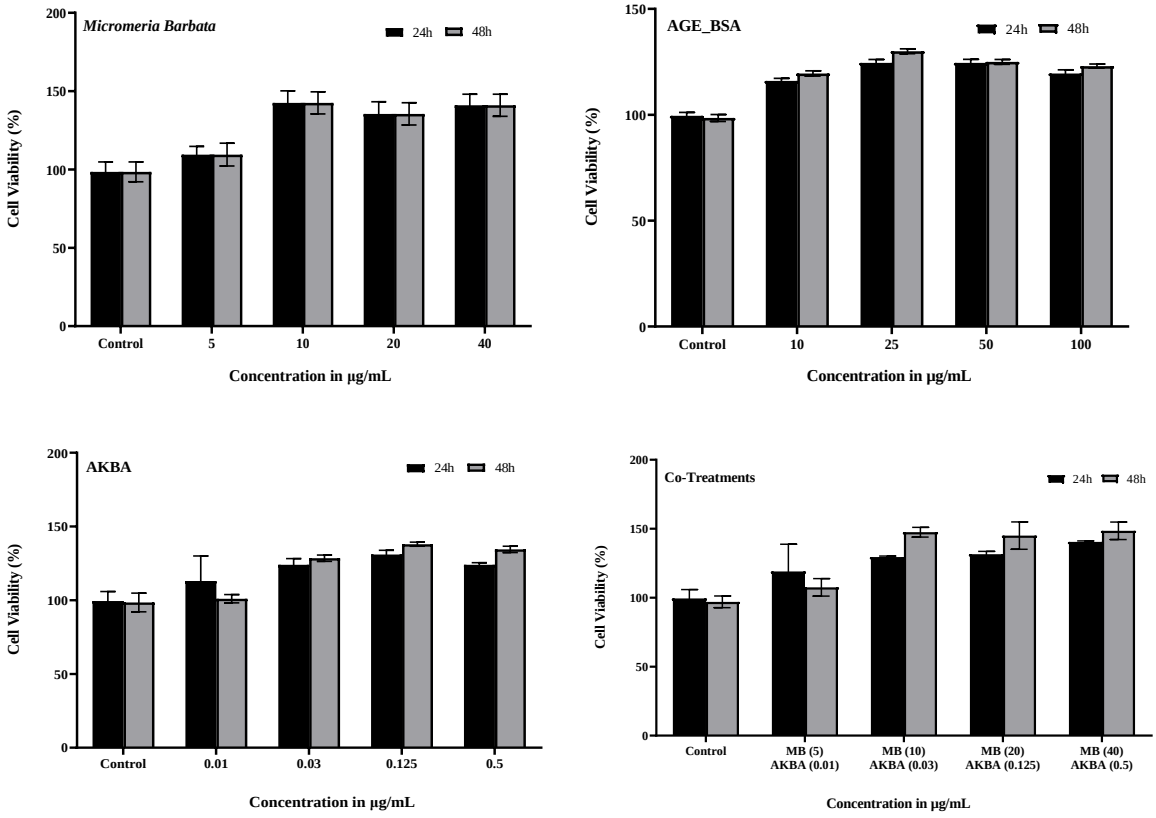

Figure 1.
Cytotoxicity and cell viability.

PBMC cells were subjected to the cytotoxic effects of MB-ethanolic extract (MB), AGE-BSA, AKBA, and binary mixtures of (AKBA–MB) at different concentrations. Cell viability was determined by MTT assay. Data are expressed as the mean ± SEM of triplicate samples and as absorbance units normalized to values in untreated cells (100%).

3.2. Effects of Age on Pro-Inflammatory Cytokine Gene Levels in PBMC

Cells were exposed to two doses of AGE-BSA (10 and 25 µg/ml) over two different time points (6 and 24 hours) to determine the optimal incubation conditions that would elicit cell signaling responses identical to the positive control, PHA (5 µg/mL), a non-specific stimulator of T-cells. After 6 and 24 hours, there was a significant rise ($p<0.05$) in the gene levels of pro-inflammatory mediators *TNF-α*, *IL-6*, and *IL-1β*, as shown in Figure 2. *TNF-α* significantly increased 0.7-fold and 0.6-fold when treated with AGE-BSA at 10 µg/ml at 6 and 24 hours, respectively. The expression levels remained comparable to those of cells cultured in media at 25 µg/mL of AGE-BSA. After 6 hours of incubation with AGE-BSA at 10 µg/mL, gene expression increased by 100-fold for *IL-6* and 5.5-fold for *IL-1β*. After 24 hours with AGE-BSA (10 µg/ml), gene expression showed a further significant increase to 115-fold for *IL-6* and 11.7-fold for *IL-1β* ($p<0.05$). At 25 µg/ml, gene expression remained significantly elevated at the two time points, showing a 104-fold and 5-fold increase for *IL-6* and *IL-1β*, respectively, at 6 hours and a 99-fold and 10.86-fold increase for *IL-6* and IL-1β, respectively, at 24 hours. The increase of the three cytokines *TNF-α*, *IL-6*, and *IL-1β* post AGE-BSA treatment, with no effect observed on the anti-inflammatory IL-4, indicates that AGE-BSA generates inflammatory responses in PBMC. The results suggest that AGE-BSA exposure may contribute to the development of deleterious effects by altering cytokine expression and increasing inflammation.

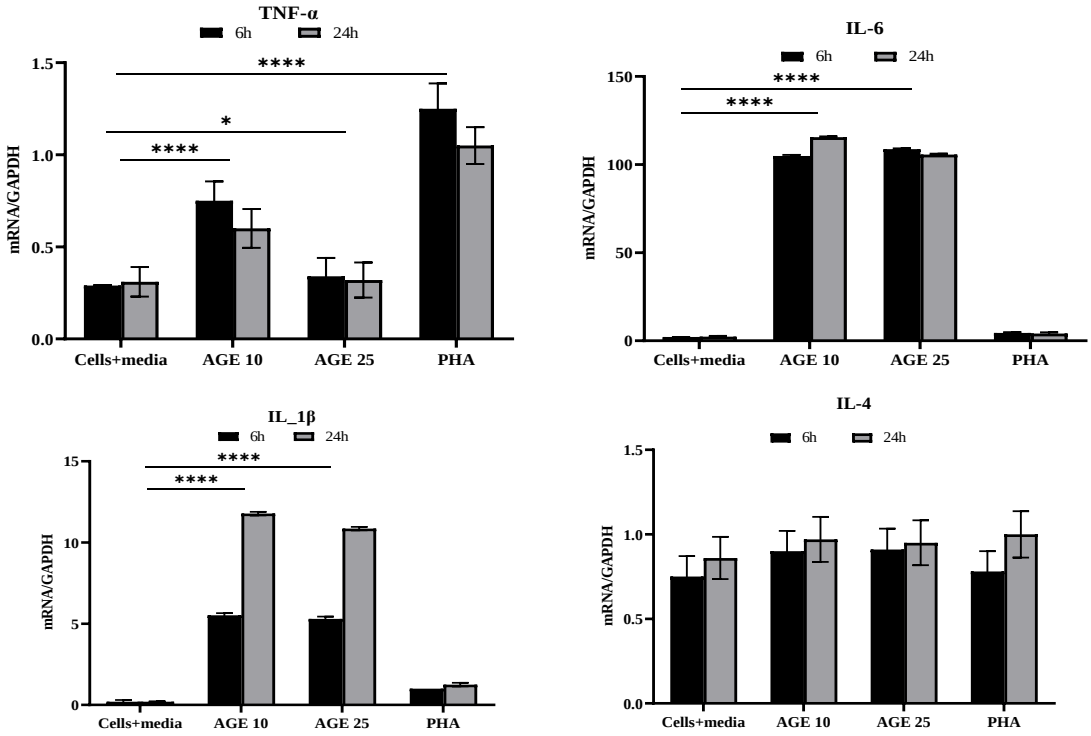

Figure 2.
Time course of pro-inflammatory cytokine levels in PBMC treated with 10 or 25 µg/mL of AGE-BSA.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

The representative RT-PCR analysis demonstrates the impact of PHA (positive control, 6- and 24-hour incubation) and 10 or 25 g/mL of AGE-BSA on the levels of inflammatory cytokines in the PBMC cell line after 6 and 24 hours of incubation, in comparison to the untreated cells (cell media). Data are expressed as the mean ± SEM of triplicate samples. **p<0.01, *p<0.005.

3.3. Effects of AGE-BSA on Pro-Inflammatory Cytokines Gene Level in THP-1 Cells

THP-1 cells were incubated with two different concentrations of AGE-BSA (10 and 25 μg/ml) for 6 and 24 hours. LPS (1 μg/ml) was used as a positive control. Subsequently, a noteworthy increase ($p<0.05$) in the gene expression levels of three pro-inflammatory mediators, *TNF-α*, *IL-6*, and *IL-1β*, was observed, as depicted in Figure 3. After 6 hours of treatment with AGE-BSA at a concentration of 10 μg/ml, gene expression increased significantly: 7.3 times for *TNF-α*, 325 times for *IL-6*, and 11 times for *IL-1β* ($p<0.05$). At a higher concentration of 25 μg/ml, gene expression remained significantly elevated, 6-fold for *TNF-α*, 295-fold for *IL-6*, and 9.1-fold for *IL-1β*. After 24 hours, upon treatment with AGE-BSA at a concentration of 10 μg/ml, the gene expression increased significantly for *TNF-α* to be 7.7-fold, while it decreased significantly for *IL-6* and *IL-1β* to be 37-fold and 2.3-fold, respectively. At the higher concentration of 25 μg/ml, gene expression of *TNF-α* remained 6-fold, while it decreased to 38-fold for *IL-6* and 1.9-fold for *IL-1β*.

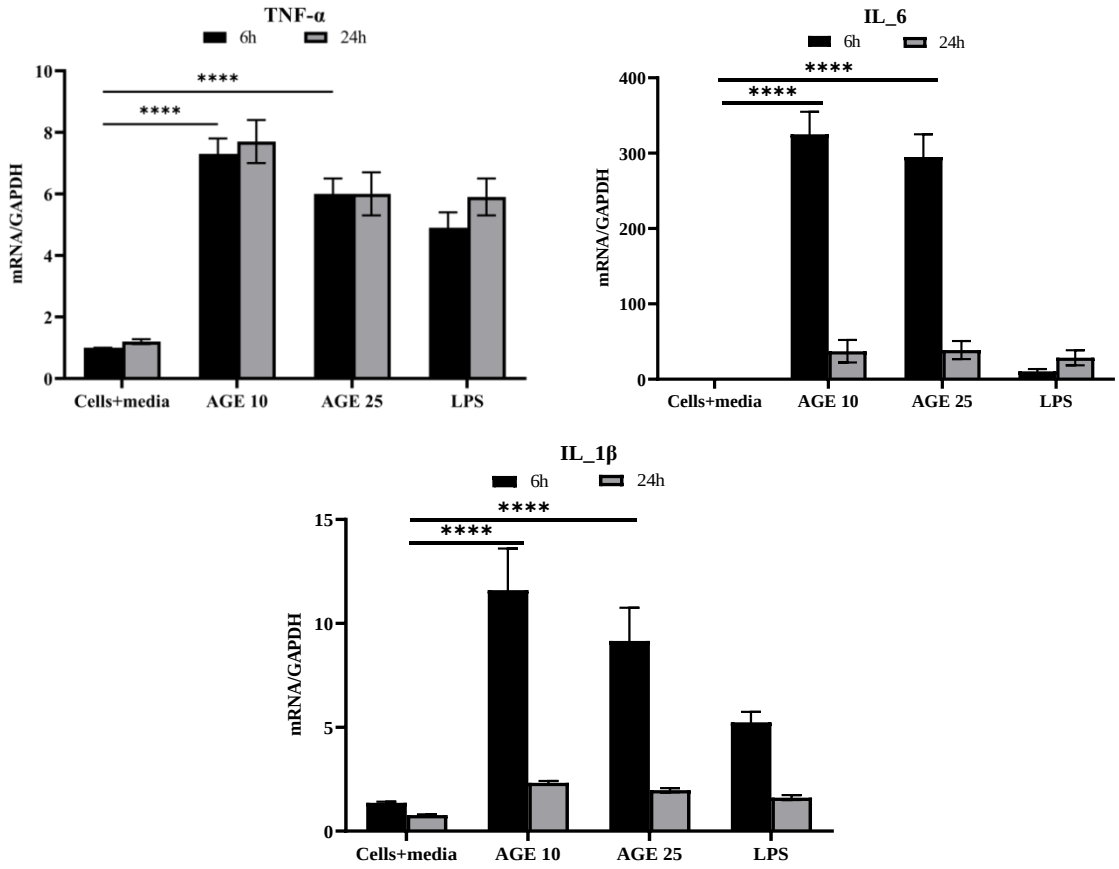

Figure 3.
Pro-inflammatory cytokine levels in the THP-1 cell line treated with 10 or 25 μg/mL of AGE-BSA.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

The representative RT-PCR analysis demonstrates the impact of LPS (positive control, 6- and 24-hour incubation) and 10 or 25 µg/mL of AGE-BSA on *TNF-α*, *IL-6*, and *IL-1β* levels in the THP-1 cell line after 6 hours of incubation compared with the untreated cells (cell media). Data are expressed as the mean ± SEM of triplicate samples. ****p<0.0001.

3.4. Effect of MB and AKBA Treatment on the Gene Expression of the Pro-Inflammatory Mediators TNF-α, IL-6, and IL-1β in PBMC and THP-1 Cells

The gene expression of *TNF-α*, *IL-6*, and *IL-1β* was measured in PBMC and THP-1 pre-incubated for 18 hours with two different concentrations of either MB (5 and 40 µg/ml) or AKBA (0.027 and 0.3 µg/ml), followed by a 6-hour stimulation with AGE-BSA (10 µg/ml). It is essential to emphasize that our study is the first to investigate the anti-inflammatory effect of MB. However, earlier research has explored the potential anti-inflammatory impact of AKBA, but not in the AGE-BSA context.

3.5. Dose-Dependent Effects of MB and AKBA on TNF-α Gene Expression in PBMC and THP-1 Cells

PBMC and THP-1 cells treated with two concentrations of MB, 5 and 40 µg/mL, led to a notable down-regulation of the gene-level expression of *TNF-α* (Figure 4). In PBMCs, both doses of MB significantly decreased gene expression. MB at 40 µg/mL showed a more substantial decrease than 5 µg/mL (0.3-fold and 0.8-fold, respectively). However, in THP-1 cells, MB at 40 µg/ml induced a significant decline compared to 5 µg/ml (0.2-fold and 0.47-fold, respectively) ($p<0.05$). Similarly, AKBA at 0.027 and 0.3 µg/mL causes a significant reduction in PBMC and THP-1 cells. In the case of PBMC, two doses of AKBA have a marked and significant decrease, reaching as low as 0.66 and 0.55-fold (0.027 and 0.3 µg/mL, respectively; $p<0.05$). In THP-1 cells, AKBA at 0.027 and 0.3 µg/mL dosages causes down-regulation to reach 0.98-fold and 0.46-fold, respectively ($p<0.05$). These results reveal the anti-inflammatory effect of both MB and AKBA on *TNF-α* gene expression in PBMC and THP-1 cells.

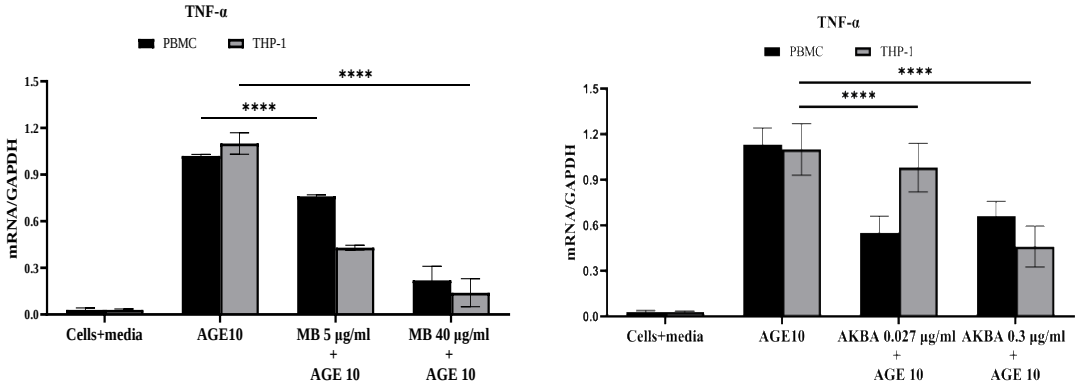

Figure 4.
Analysis of MB-ethanolic extract and AKBA on *TNF-α* gene expression in AGE-BSA-treated PBMC and the THP-1 cell line.

PBMC and THP-1 cells were preincubated for 18 hours with different doses of MB or AKBA before being stimulated for 6 hours with AGE-BSA (10 µg/mL). RT-PCR was used to investigate the inhibitory impact of MB ethanolic and AKBA on TNF-α expression. Data are expressed as the mean ± SEM of triplicate samples.

3.6. Dose-Dependent Effect of MB and AKBA on IL-6 Gene Expression in PBMC and THP-1

In PBMC and THP-1 cells, stimulating with a 10 µg/mL AGE-BSA dose resulted in a significant increase to 1-fold and 1.2-fold in *IL-6* expression, respectively, as shown in Figure 5. Treating PBMC

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

and THP-1 cells with two concentrations of MB (5 and 40 µg/mL) caused a significant ($p<0.05$) reduction in *IL-6* gene expression levels. In PBMCs, both doses of MB significantly decreased gene expression. MB at 40 µg/mL reverted *IL-6* production to baseline, showing a more substantial decrease than 5 µg/mL (0.2-fold and 0.7-fold, respectively). Similarly, in THP-1 cells, MB at 40 µg/mL caused a significant decline compared to 5 µg/mL (0.1-fold and 0.4-fold, respectively). AKBA treatment also substantially reduces the *IL-6* production in both cell types at dosages of 0.027 and 0.3 µg/ml. AKBA treatments result in an approximate decrease of 0.7-fold with both doses across the two cell types. These data highlight the ability of both MB and AKBA to reduce *IL-6*-mediated pro-inflammatory responses effectively.

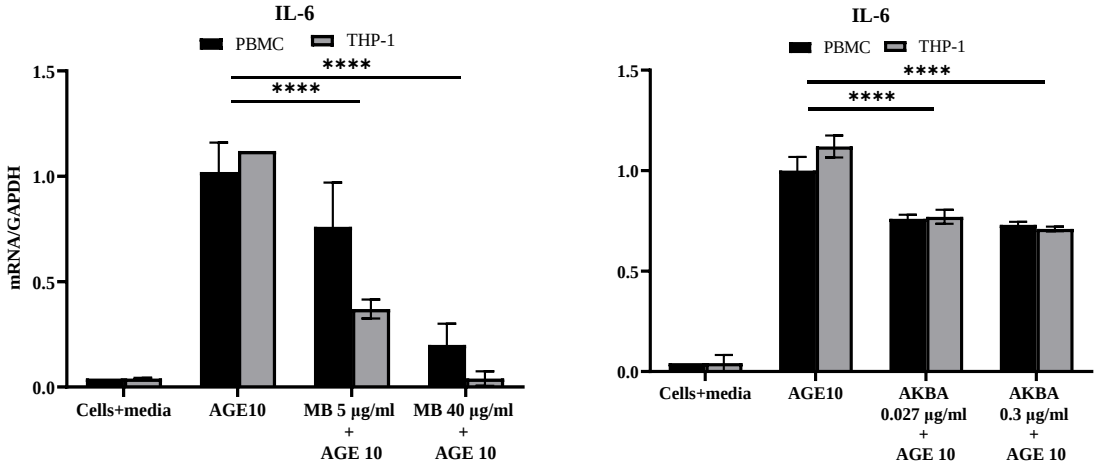

Figure 5.
MB-ethanolic extract and AKBA influence the gene expression study of *IL-6* in AGE-BSA-treated PBMC and the THP-1 cell line.

PBMC and THP-1 cells were pretreated with increasing doses of MB or AKBA for 18 hours before stimulation with AGE-BSA (10 µg/mL) for 6 hours.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

We investigated the inhibition of IL-6 expression by MB and AKBA using RT-PCR. Data are expressed as the mean ± SEM of triplicate samples.

IL-1β gene expression in PBMC and THP-1 cells treated with MB and AKBA in a dose-dependent manner

PBMC and THP-1 cells showed a significant increase in *IL-1*β expression following AGE-BSA (10 µg/ml) stimulation. PBMC and THP-1 cells treated with two concentrations of MB (5 and 40 µg/mL) exhibited a decrease in *IL-1*β gene expression to 0.7-fold and 0.19-fold, respectively ($p < 0.05$) (Figure 6). Similarly, in THP-1 cells, MB caused significant changes at 5 µg/mL and 40 µg/mL, reducing gene levels to 0.72-fold and 0.75-fold. AKBA treatment inhibited expression in PBMCs, decreasing it by approximately 0.6-fold at 0.027 µg/mL and 0.7-fold at 0.3 µg/mL ($p < 0.05$). In THP-1 cells, at 0.027 µg/mL or 0.3 µg/mL, a significant fold decrease to 0.6-fold was observed ($p < 0.05$).

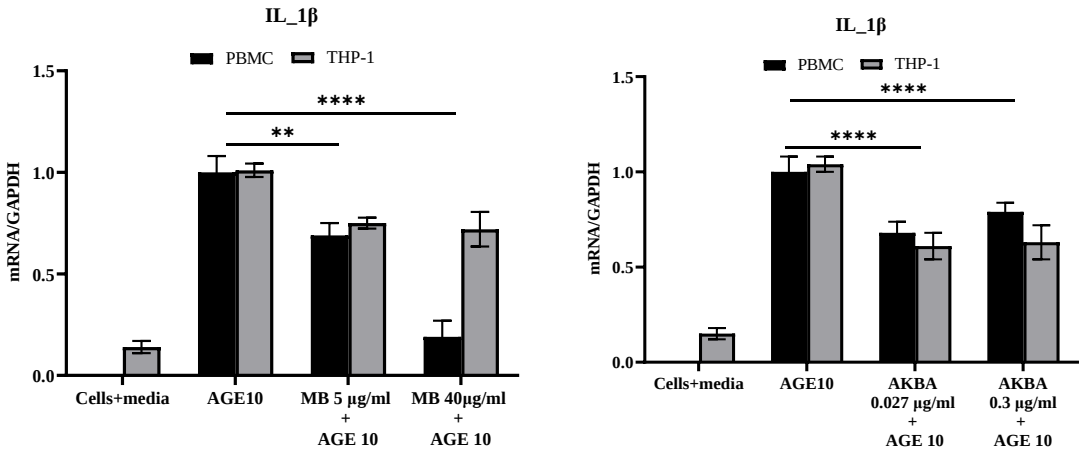

Figure 6.
Analysis of MB-ethanolic extract and AKBA on *IL-1β* gene expression in AGE-BSA-treated PBMC and the THP-1 cell line. Data are expressed as the mean ± SEM of triplicate samples.

4. Discussion

AGEs trigger pro-inflammatory effects by interacting with RAGE, which activates NF-κB and boosts inflammatory responses [37]. The AGEs-RAGE axis works with toll-like receptor (TLR) signaling pathways to connect innate immunity and problems with the metabolic system [17]. This association elucidates the persistence of low-grade inflammation in cardio-metabolic disorders, including diabetes and atherosclerosis. Previous studies have demonstrated the role of AGE in modulating acute inflammation in macrophages [38] and in augmenting the synthesis of pro-inflammatory cytokines (IL-6 and TNF-α) [39, 40]. This study adds a unique perspective to the existing literature by examining the anti-inflammatory effects of Lebanese MB ethanolic extract and AKBA within the context of AGE-induced inflammation for the first time. Both compounds were evaluated for safety prior to their use in cell models. None of the tested concentrations (MB: 5, 10, 20, and 40 µg/mL; AKBA: 0.01, 0.03, 0.125, and 0.5 µg/mL) or durations (24 and 48 hours) showed signs of cytotoxicity. These findings confirm that the observed anti-inflammatory effects were not due to cytotoxic stress but rather genuine bioactivity. Additionally, the concentrations chosen for MB (5 and 40 µg/mL) were guided by preliminary cytotoxicity screening conducted in our laboratory (unpublished data), and the dosing recommendations were based on those internal findings. For AKBA, the selected concentrations were 0.027 and 0.3 µg/mL, with the initial concentration of 0.027 µg/mL derived from previous studies demonstrating its biological activity at sub-micromolar levels [41]. We subsequently evaluated an elevated concentration of 0.3 µg/mL, which exhibited no cytotoxic effects under our experimental

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

conditions; therefore, both doses were carried forward for downstream mechanistic assays. Consequently, we performed trials to assess two concentrations of AGE-BSA (10 and 25 μg/mL) and chose 10 μg/mL for our study. This concentration was derived from empirical evidence showing that at 10 μg/mL of AGE-BSA, pro-inflammatory cytokines were increased at all dosages. Lowering the dosage to 25 μg/mL, however, caused a down-regulation in TNF-α levels in the PBMC. Hence, it is justified that we use 10 μg/mL as the AGE-BSA concentration for this research. At 10 μg/mL AGE-BSA, the pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) were significantly increased at the 6- and 24-hour time points, in agreement with other works [42, 43]. IL-4, an anti-inflammatory cytokine with pleiotropic effects [44], showed no effect. The absence of IL-4 modulation in this study is significant because, in other studies, AGEs tend to promote macrophages to adopt a pro-inflammatory M1 phenotype while decreasing cytokines of M2 macrophages, such as IL-4 and IL-10 [45]. Hence, evidence for the hypothesis that AGEs create proteolytic, inflammatory signaling mechanisms to sustain is increased.

As AGE-BSA levels continued to increase, the levels of TNF-α production started to decrease. A biphasic curve pattern was observed, where decreased inflammation at higher levels of AGE saturation suggests cellular stress, which triggers the shutdown of inflammation. This shutdown process could be due to self-regulatory internal feedback loops, which implicate the suppressor of cytokine signaling family [46]. These differences demonstrate the importance of accurately defining the dose and exposure time when reproducing physiological levels of AGE in vitro.

According to the findings, TNF-α, IL-6, and IL-1β were successfully downregulated in PBMC and THP-1 cells by both MB and AKBA. AKBA showed much greater potency per unit mass compared to MB in terms of relative potency. In contrast to mid-microgram to tens-of-microgram values for MB (5 and 40 μg/mL), the observed decreases in cytokine expression after AKBA treatment occurred at concentrations in the low-microgram range (0.027 and 0.3 μg/mL). This indicates that MB's anti-inflammatory action is probably mediated by many phytochemicals working in concert, while AKBA, a highly bioactive, pure boswellic acid, acts more precisely pharmacologically at lower dosages. Therefore, MB provides wider multi-component modulation consistent with whole-extract phytochemistry, but AKBA is more powerful on a μg-for-μg basis.

Furthermore, the data suggest that there was a progressive, positive, dose-dependent decrease as concentrations of MB were increased to a maximum level of 40 μg/ml, which resulted in the greatest decrease in both cell types tested. It's noteworthy to mention that our study is the first to investigate the dosage of MB ethanolic extract in AGE-induced inflammatory models utilizing PBMC and THP-1 cells, with concentrations ranging from 5 to 40 μg/mL. Because no comparable dose-response studies have been published, there is no clear standard for direct quantitative comparison. Thus, our study develops preliminary reference concentrations rather than expanding on existing established values.

AKBA achieved comparable TNF-α modulation, which further strengthens its ability to control the process of inflammation at a significantly lower dose of 0.3μg/ml. These findings align with previous reports, yet the dose-dependent details indicate a need for deeper examination. TNF-α levels decreased with MB at 40μg/ml and with AKBA at a considerably lower dose of 0.3μg/ml, indicating a significant anti-inflammatory effect for both compounds. MB at 40μg/ml was most effective for dose-dependent IL-6 reduction in THP-1 and PBMC cells. In the case of AKBA, IL-6 was suppressed in PBMC and THP-1 cells at both tested concentrations. These results show MB has anti-inflammatory activity, especially targeting IL-6, a key cytokine in pro-inflammatory signaling and immune response orchestration [46, 47].

The ongoing inhibition of IL-6 by AKBA at all levels is worth further exploration due to IL-6's known associations with metabolic perturbations, chronic inflammation, and immune imbalance [48]. This may suggest that AKBA acts by persistently diminishing the metabolic inhibition of other, likely upstream, transcription factors such as STAT3 and NF-κB, thereby decreasing the maturation and secretion of IL-6 through NF-κB inhibition [30, 49]. This is likely the rationale behind the lower levels

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

of IL-6 seen in the experimental models. The Lebanese MB ethanolic extract is rich in various bioactive phytochemicals [45, 50] and is known to have considerable anti-inflammatory and antioxidant activities. The Lebanese MB strain's essential oil, as previously reported, has antimicrobial and antifungal activities that may have therapeutic applications [23, 50]. This particular study further enriches the current literature by being the first to demonstrate that MB has the potential to mitigate AGE-induced inflammatory responses and thereby adds further evidence to the therapeutic applications of MB. Both MB and AKBA were able to show a reduction of IL-1β expression in both cell types as a dose-dependent response in PBMCs, while in the THP-1 cells, this was not observed. The differences in the thresholds for inflammasome activation might serve to explain the non-linear pattern of IL-1β expression observed in the THP-1 cells. The differences in the response of IL-1β in the THP-1 cells suggest a potential for non-homogeneous activation of the inflammasome in these cells. In contrast to primary PBMCs, there is often a cell line of monocytes, such as the THP-1 cell line, that demonstrates a diverse pattern of NLRP3 priming, resulting in changes to the NLRP3 as well as the cytokine responses to inflammatory signals [51].

Each of these cellular phenomena also has separate functions in the immune system. While PBMCs mainly perform primary immune responses that activate multiple cells, THP-1 cells can reprogram the intracellular signaling pathways that govern the timing of cytokine release and receptor expression [52, 53]. Given such systemic heterogeneity, it is generally difficult to establish clear dose-response relationships in vitro; cellular context can radically alter outcomes. Of the various cell systems, IL-1β suppression was the only outcome that MB was able to influence. Beyond the suppression of caspase-1, the master protease for the release of IL-1β and a crucial modulator of inflammation, this shows more complexity at work, perhaps the manipulation of many inflammatory pathway targets [54]. Several plant compounds have been shown to exert radical modulation of inflammation through the regulation of oxidative stress and inhibition of NF-κB [39, 40]. NF-κB suppression as a result of oxidative stress inhibition reinforces the idea that MB and AKBA act by the same anti-inflammatory pathway. Wang et al. [30] suggest AKBA also alters the pro- and anti-inflammatory balance of macrophages, shifting it toward an anti-inflammatory state, as does the inhibition of NF-κB signaling. These collective observations characterize MB and AKBA as controlling the redox and immune homeostasis of the organism, as factors that stabilize the organism's internal environment well beyond neutralizing one inflammatory signal.

5. Conclusions and Limitations

In summary, the findings showed that the ethanolic extract of MB and AKBA modulates the gene expression of key pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) in AGE-BSA–stimulated PBMC and THP-1 cells. These findings imply possible anti-inflammatory effects at the transcriptional level, laying the groundwork for future research into protein expression and functional implications. Given their natural origin and redox-modulating characteristics, MB extract and AKBA may be viable options for treating AGE-related inflammation in metabolic and inflammatory illnesses, including diabetes. Further investigation into protein expression, molecular validation, and in vivo models is necessary to substantiate these medical implications. This study is limited to the IL-6, IL-1β, and TNF-α profile; thus, consideration of other inflammatory mediators would be valuable in elucidating the mechanisms at play. An analytical assessment is necessary to identify the elements responsible for the differences observed in some cytokines in the various cell lines. This study captures gene expression at 6 and 24 hours; however, these time points may not encompass the full spectrum of molecular changes associated with chronic inflammation. This work will ultimately require supportive in vivo and clinical studies. This study would confirm the clinical value of the investigated substances, their ability to interact with other treatments, and their long-term safety.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 2: 340-354, 2026
DOI: 10.55214/2576-8484.v10i2.12061

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

References

[1] A. Twarda-Clapa, A. Olczak, A. M. Białkowska, and M. Koziołkiewicz, "Advanced glycation end-products (AGEs): Formation, chemistry, classification, receptors, and diseases related to AGEs," *Cells*, vol. 11, no. 8, p. 1312, 2022. https://doi.org/10.3390/cells11081312

[2] M. Khalid, G. Petroianu, and A. Adem, "Advanced glycation end products and diabetes mellitus: Mechanisms and perspectives," *Biomolecules*, vol. 12, no. 4, p. 542, 2022. https://doi.org/10.3390/biom12040542

[3] C. Liu *et al.*, "Innate immune cell profiling in peripheral blood mononuclear cells of patients with moyamoya disease," *Inflammation*, vol. 48, no. 4, pp. 2444-2457, 2025. https://doi.org/10.1007/s10753-024-02201-4

[4] J. Trück *et al.*, "Pneumococcal serotype-specific antibodies persist through early childhood after infant immunization: Follow-up from a randomized controlled trial," *PLoS One*, vol. 9, no. 3, p. e91413, 2014. https://doi.org/10.1371/journal.pone.0091413

[5] V. De Mello *et al.*, "Downregulation of genes involved in NFκB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: The GENOBIN study," *Diabetologia*, vol. 51, pp. 2060-2067, 2008. https://doi.org/10.1007/s00125-008-1132-7

[6] R. K. Charlotte, *Peripheral blood mononuclear cells, in The Impact of Food Bioactives on Health: in vitro and ex vivo models, K. Verhoeckx, P. Cotter, and I. López-Expósito, Eds.* Cham (CH): Springer, 2015.

[7] M. Pietrzak, M. Chaszczewska-Markowska, and M. Zemelka-Wiacek, "Porcine peripheral blood mononuclear cells (PBMCs): Methods of isolation, cryopreservation, and translational applications in human studies," *Journal of Clinical Medicine*, vol. 14, no. 10, p. 3432, 2025. https://doi.org/10.3390/jcm14103432

[8] D. Hirayama, T. Iida, and H. Nakase, "The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis," *International Journal of Molecular Sciences*, vol. 19, no. 1, p. 92, 2017. https://doi.org/10.3390/ijms19010092

[9] D. Schenten and R. Medzhitov, "The control of adaptive immune responses by the innate immune system," *Advances in Immunology*, vol. 109, pp. 87-124, 2011. https://doi.org/10.1016/B978-0-12-387664-5.00003-0

[10] C.-Y. Shen *et al.*, "The development of maillard reaction, and advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling inhibitors as novel therapeutic strategies for patients with AGE-related diseases," *Molecules*, vol. 25, no. 23, p. 5591, 2020. https://doi.org/10.3390/molecules25235591

[11] W. Chanput, J. J. Mes, and H. J. Wichers, "THP-1 cell line: An in vitro cell model for immune modulation approach," *International Immunopharmacology*, vol. 23, no. 1, pp. 37-45, 2014. https://doi.org/10.1016/j.intimp.2014.08.002

[12] T. Takahashi and M. Miyazawa, "Potent α-glucosidase inhibitors from safflower (Carthamus tinctorius L.) seed," *Phytotherapy Research*, vol. 26, no. 5, pp. 722-726, 2012. https://doi.org/10.1002/ptr.3622

[13] I. K. Lukic, P. M. Humpert, P. P. Nawroth, and A. Bierhaus, "The RAGE pathway: Activation and perpetuation in the pathogenesis of diabetic neuropathy," *Annals of the New York Academy of Sciences*, vol. 1126, no. 1, pp. 76-80, 2008. https://doi.org/10.1196/annals.1433.059

[14] B. Moser *et al.*, "Receptor for advanced glycation end products expression on T cells contributes to antigen-specific cellular expansion in vivo," *The Journal of Immunology*, vol. 179, no. 12, pp. 8051-8058, 2007. https://doi.org/10.4049/jimmunol.179.12.8051

[15] A. P. Burke *et al.*, "Morphologic findings of coronary atherosclerotic plaques in diabetics: A postmortem study," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 7, pp. 1266-1271, 2004. https://doi.org/10.1161/01.ATV.0000131783.74034.97

[16] X. Jin *et al.*, "Advanced glycation end products enhance macrophages polarization into M1 phenotype through activating RAGE/NF-κB pathway," *BioMed Research International*, vol. 2015, no. 1, p. 732450, 2015. https://doi.org/10.1155/2015/732450

[17] A. Bierhaus *et al.*, "Understanding RAGE, the receptor for advanced glycation end products," *Journal of Molecular Medicine*, vol. 83, pp. 876-886, 2005. https://doi.org/10.1007/s00109-005-0688-7

[18] R. Donato *et al.*, "Functions of S100 proteins," *Current Molecular Medicine*, vol. 13, no. 1, pp. 24-57, 2013. https://doi.org/10.2174/156652413804486214

[19] Q. Liu, Y. Huo, H. Zheng, J. Zhao, L. Jia, and P. Wang, "Ethyl pyruvate suppresses the growth, invasion and migration and induces the apoptosis of non-small cell lung cancer cells via the HMGB1/RAGE axis and the NF-κB/STAT3 pathway," *Oncology Reports*, vol. 42, no. 2, pp. 817-825, 2019. https://doi.org/10.3892/or.2019.7176

[20] S. Vladimir-Knežević, O. Cvijanović, B. Blažeković, M. Kindl, M. B. Štefan, and R. Domitrović, "Hepatoprotective effects of Micromeria croatica ethanolic extract against CCl4–induced liver injury in mice," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, p. 233, 2015. https://doi.org/10.1186/s12906-015-0763-8

[21] S. Rawas, N. Al Hakawati, A. Mcheik, and D. E. S. El Badan, "Micromeria barbata for targeting MRSA virulence: In silico and in vitro studies," *Heliyon*, vol. 11, no. 1, p. e41536, 2025. https://doi.org/10.1016/j.heliyon.2024.e41536

[22] S. Rawas, D. El-Badan, and N. Al Hakawati, "Phytochemical analysis and antimicrobial activity of Micromeria barbata leaf and stem extracts against Pseudomonas aeruginosa: Insights from molecular docking and in vitro assays," *BAU Journal - Science and Technology*, vol. 6, no. 2, 2025. https://doi.org/10.54729/2959-331X.1149

[23] K. El Omari, M. Hamze, S. Alwan, C. Jama, and N. Chihib, "Antifungal activity of the essential oil of Micromeria barbata an endemic Lebanese Micromeria species collected at North Lebanon," *Journal of Materials and Environmental Science*, vol. 7, no. 11, pp. 4158-4167, 2016.

[24] M. Hamwi, M. Aboul Ela, and A. El-Lakany, "Genus Micromeria: A review article," *BAU Journal - Health and Wellbeing*, vol. 3, no. 2, p. Article 5, 2021. https://doi.org/10.54729/2789-8288.1130

[25] S. Pérez-Piñero *et al.*, "Efficacy of Boswellia serrata extract and/or an omega-3-based product for improving pain and function in people older than 40 Years with persistent knee pain: a randomized double-blind controlled clinical trial," *Nutrients*, vol. 15, no. 17, p. 3848, 2023. https://doi.org/10.3390/nu15173848

[26] I. V. B. Valente *et al.*, "The anti-proliferative effects of a frankincense extract in a window of opportunity phase ia clinical trial for patients with breast cancer," *Breast Cancer Research and Treatment*, vol. 204, pp. 521-530, 2024. https://doi.org/10.1007/s10549-023-07215-4

[27] A. Ahangarpour *et al.*, "Effect of Boswellia serrata supplementation on blood lipid, hepatic enzymes and fructosamine levels in type2 diabetic patients," *Journal of Diabetes & Metabolic Disorders*, vol. 13, no. 1, p. 29, 2014. https://doi.org/10.1186/2251-6581-13-29

[28] A. S. Sayed and N. S. E. D. El Sayed, "Co-administration of 3-acetyl-11-keto-beta-boswellic acid potentiates the protective effect of celecoxib in lipopolysaccharide-induced cognitive impairment in mice: possible implication of anti-inflammatory and antiglutamatergic pathways," *Journal of Molecular Neuroscience*, vol. 59, pp. 58-67, 2016. https://doi.org/10.1007/s12031-016-0734-7

[29] C. Wei *et al.*, "Acetyl-11-keto-β-boswellic acid ameliorates cognitive deficits and reduces amyloid-β levels in APPswe/PS1dE9 mice through antioxidant and anti-inflammatory pathways," *Free Radical Biology and Medicine*, vol. 150, pp. 96-108, 2020. https://doi.org/10.1016/j.freeradbiomed.2020.02.022

[30] Y. Wang *et al.*, "Acetyl-11-keto-beta-boswellic acid modulates macrophage polarization and Schwann cell migration to accelerate spinal cord injury repair in rats," *CNS Neuroscience & Therapeutics*, vol. 30, no. 3, p. e14642, 2024. https://doi.org/10.1111/cns.14642

[31] R. Morisi *et al.*, "Growth inhibition of medullary thyroid carcinoma cells by pyrazolo-pyrimidine derivates," *Journal of Endocrinological Investigation*, vol. 30, pp. RC31-RC34, 2007. https://doi.org/10.1007/BF03349220

[32] D. Karakaxas *et al.*, "Genetic polymorphisms of interleukin 1β gene and sporadic pancreatic neuroendocrine tumors susceptibility," *World Journal of Gastrointestinal Oncology*, vol. 8, no. 6, pp. 520-525, 2016. https://doi.org/10.4251/wjgo.v8.i6.520

[33] M. Figueiredo, C. Salter, A. Andrietti, M. Vandenplas, D. Hurley, and J. Moore, "Validation of a reliable set of primer pairs for measuring gene expression by real-time quantitative RT-PCR in equine leukocytes," *Veterinary Immunology and Immunopathology*, vol. 131, no. 1-2, pp. 65-72, 2009. https://doi.org/10.1016/j.vetimm.2009.03.013

[34] S. Saini, H. Singha, P. Siwach, and B. Tripathi, "Recombinant horse interleukin-4 and interleukin-10 induced a mixed inflammatory cytokine response in horse peripheral blood mononuclear cells," *Veterinary World*, vol. 12, no. 4, pp. 496-503, 2019. https://doi.org/10.14202/vetworld.2019.496-503

[35] M. Saxena, D. Ali, D. R. Modi, M. H. Almarzoug, S. Hussain, and S. Manohrdas, "Association of TNF-α gene expression and release in response to anti-diabetic drugs from human adipocytes in vitro," *Diabetes, Metabolic Syndrome and Obesity*, vol. 13, pp. 2633-2640, 2020. https://doi.org/10.2147/DMSO.S265362

[36] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method," *Methods*, vol. 25, no. 4, pp. 402-408, 2001. https://doi.org/10.1006/meth.2001.1262

[37] D. Catanzaro *et al.*, "Boswellia serrata preserves intestinal epithelial barrier from oxidative and inflammatory damage," *PloS One*, vol. 10, no. 5, p. e0125375, 2015. https://doi.org/10.1371/journal.pone.0125375

[38] Q. Yue, Y. Song, Z. Liu, L. Zhang, L. Yang, and J. Li, "Receptor for advanced glycation end products (RAGE): A pivotal hub in immune diseases," *Molecules*, vol. 27, no. 15, p. 4922, 2022. https://doi.org/10.3390/molecules27154922

[39] Q. Qin, J. Niu, Z. Wang, W. Xu, Z. Qiao, and Y. Gu, "Astragalus membranaceus inhibits inflammation via phospho-P38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways in advanced glycation end product-stimulated macrophages," *International Journal of Molecular Sciences*, vol. 13, no. 7, pp. 8379-8387, 2012. https://doi.org/10.3390/ijms13078379

[40] R. K. Perkins *et al.*, "Advanced glycation end products and inflammatory cytokine profiles in maintenance hemodialysis patients after the ingestion of a protein-dense meal," *Journal of Renal Nutrition*, vol. 33, no. 1, pp. 181-192, 2023. https://doi.org/10.1053/j.jrn.2021.11.006

[41] C.-Y. Shen *et al.*, "Advanced glycation end products of bovine serum albumin suppressed Th1/Th2 cytokine but enhanced monocyte IL-6 gene expression via MAPK-ERK and MyD88 transduced NF-κB p50 signaling pathways," *Molecules*, vol. 24, no. 13, p. 2461, 2019. https://doi.org/10.3390/molecules24132461

[42] R. Kokkola *et al.*, "RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages," *Scandinavian Journal of Immunology*, vol. 61, no. 1, pp. 1-9, 2005. https://doi.org/10.1111/j.0300-9475.2005.01534.x

[43] H. Yonekura, Y. Yamamoto, S. Sakurai, T. Watanabe, and H. Yamamoto, "Roles of the receptor for advanced glycation endproducts in diabetes-induced vascular injury," *Journal of Pharmacological Sciences*, vol. 97, no. 3, pp. 305-311, 2005. https://doi.org/10.1254/jphs.CPJ04005X

[44] A. A. Al-Qahtani, F. S. Alhamlan, and A. A. Al-Qahtani, "Pro-inflammatory and anti-inflammatory interleukins in infectious diseases: A comprehensive review," *Tropical Medicine and Infectious Disease*, vol. 9, no. 1, p. 13, 2024. https://doi.org/10.3390/tropicalmed9010013

[45] Y. H. Gonfa *et al.*, "Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review," *Current Research in Biotechnology*, vol. 6, p. 100152, 2023. https://doi.org/10.1016/j.crbiot.2023.100152

[46] M. Aliyu *et al.*, "Interleukin-6 cytokine: An overview of the immune regulation, immune dysregulation, and therapeutic approach," *International Immunopharmacology*, vol. 111, p. 109130, 2022. https://doi.org/10.1016/j.intimp.2022.109130

[47] Y. Luo and S. G. Zheng, "Hall of fame among pro-inflammatory cytokines: interleukin-6 gene and its transcriptional regulation mechanisms," *Frontiers in Immunology*, vol. 7, p. 604, 2016. https://doi.org/10.3389/fimmu.2016.00604

[48] C. A. Hunter and S. A. Jones, "IL-6 as a keystone cytokine in health and disease," *Nature Immunology*, vol. 16, pp. 448-457, 2015. https://doi.org/10.1038/ni.3153

[49] S. Roy *et al.*, "Human genome screen to identify the genetic basis of the anti-inflammatory effects of Boswellia in microvascular endothelial cells," *DNA and Cell Biology*, vol. 24, no. 4, pp. 244-255, 2005. https://doi.org/10.1089/dna.2005.24.244

[50] Y. Bakkour, S. Alwan, H. Soufi, N. El-Ashi, M. Tabcheh, and F. El Omar, "Chemical composition of essential oil extracted from Micromeria Barbata growing in Lebanon and their antimicrobial and antioxidant properties," *Journal of Natural Products*, vol. 5, pp. 116-120, 2012.

[51] A. Gritsenko *et al.*, "Priming is dispensable for NLRP3 inflammasome activation in human monocytes in vitro," *Frontiers in Immunology*, vol. 11, p. 565924, 2020. https://doi.org/10.3389/fimmu.2020.565924

[52] H. J. Kim, D. Yang, and J. H. Hong, "Various cellular components and its signaling cascades through the involvement of signaling messengers in keratinocyte differentiation," *Antioxidants*, vol. 14, no. 4, p. 426, 2025. https://doi.org/10.3390/antiox14040426

[53] X. Li, M. Shao, X. Zeng, P. Qian, and H. Huang, "Signaling pathways in the regulation of cytokine release syndrome in human diseases and intervention therapy," *Signal Transduction and Targeted Therapy*, vol. 6, p. 367, 2021. https://doi.org/10.1038/s41392-021-00764-4

[54] Y. Li and Q. Jiang, "Uncoupled pyroptosis and IL-1β secretion downstream of inflammasome signaling," *Frontiers in Immunology*, vol. 14, p. 1128358, 2023. https://doi.org/10.3389/fimmu.2023.1128358