ORIGINAL ARTICLE

# Immunomodulatory Activity of *Moringa Oleifera* Lam. Leaf Ethanol Extract on BV2 Cell Line

Syaza Alya Najwa Syamsul Effendie[1], Latifah Saiful Yazan[1], Ramesh Ranggasamy[2,3], Rajesh Ramasamy[3]

[1] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
[2] Unit Fitokimia, Pusat Penyelidikan Perubatan Herba (HMRC),Institut Penyelidikan Perubatan (IMR),Tingkat 5, Blok C7 Institut Kesihatan Negara (NIH)
[3] Stem Cell and Immunomodulation Research Group, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor

**ABSTRACT**

**Introduction:** *Moringa oleifera*, known as Kelor in Malay and Murunggai in Tamil, is widely recognised for its nutritional and therapeutic benefits. Its leaves contain bioactive compounds such as flavonoids and polyphenols that confer antioxidant, anti-inflammatory, and anticancer properties. Modulating the unwanted inflammatory responses of microglia has become a key strategy in slowing neuroinflammation and the progression of neurodegenerative diseases. **Objectives:** This study aimed to evaluate the effects of *Moringa oleifera* ethanol extract (MoETE) on the pro-inflammatory responses of BV2 microglial cells. **Methods:** Cytotoxicity was determined using the MTT assay. The immunomodulatory potential of MoETE was assessed by measuring nitric oxide (NO) production and apoptosis in BV2 cells stimulated with 1 µg/mL LPS, using the Griess assay and flow cytometry, respectively. Experiments were performed in triplicate (n = 3). Data were analysed using one-way ANOVA followed by Dunnett's and Sidak's post hoc tests. **Results:** MoETE supported BV2 viability between 0.1–100 µg/mL but significantly reduced viability at 1000 µg/mL (46.44%, $p < 0.0001$). NO levels decreased significantly ($p < 0.05$) in LPS-stimulated cells treated with low MoETE concentrations (0.1–100 µg/mL) compared to controls, whereas higher concentrations (200–1000 µg/mL) restored NO production and induced apoptosis. **Conclusion:** MoETE demonstrates dose-dependent effects on microglial cells, exhibiting anti-inflammatory and neuroprotective potential at low concentrations but cytotoxicity and pro-inflammatory effects at higher doses. These findings suggest that controlled low-dose MoETE may beneficially modulate microglial activity to mitigate neuroinflammation.
*Malaysian Journal of Medicine and Health Sciences* (2026) 22(1): 1491.1-1491.9.doi:10.47836/mjmhs.v22.i1.1491

**Keywords:** *Moringa oleifera*, Immunomodulation, BV2 cell line, NO, Apoptosis

**Corresponding Author:**
Rajesh Ramasamy, PhD
Email: rajesh@upm.edu.my
Tel: +603-9769 2377

## INTRODUCTION

In Malaysia, a key aspect of its tradition and ethnopharmacological heritage is the use of medicinal plants to treat health conditions. These natural remedies have been historically relied upon to address various health issues, providing cost-effective and culturally accepted alternatives to conventional treatments (1–2). Among these medicinal plants recognised for their therapeutic benefits is *Moringa oleifera*, locally called "Kelor" in Malay and "Murunggai" in Tamil. This plant has gained global recognition not only for its nutritional content but also for its wide-ranging pharmacological properties (3). Notably, every part of the *M. oleifera* plant—including the leaves, flowers, pods, seeds, roots, and bark—contains unique combinations of vitamins, minerals, and bioactive compounds, enabling a broad spectrum of biological activities, such as antioxidant, anti-inflammatory, anti-diabetic, anti-hypertensive, anti-cancer, and antimicrobial effects (3–4).

Among these parts, the leaves are particularly valuable and have garnered increasing pharmacological interest. The leaves of *M. oleifera* act as a container of vital micronutrients, such as vitamins A, C, and E, calcium, zinc, and magnesium, alongside a wide range of phytochemicals including flavonoids, polyphenols, tannins, alkaloids, saponins, phenolic acids, glucosinolates, and isothiocyanates (5–6). These compounds collectively contribute to its antioxidant, anti-inflammatory, and anti-cancer properties (6). In

traditional medicine, the leaves have been used to treat inflammatory conditions and improve immune function (7). Scientific studies have validated these benefits, showing that many phytochemicals exert immunomodulatory effects by influencing intracellular signalling pathways like nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) (8,11). Through these pathways, *M. oleifera* can suppress the production of inflammatory mediators, enhance antioxidant defences, and potentially promote immunological balance by guiding immune responses towards a protective, anti-inflammatory state (8).

The immunomodulatory potential of *Moringa oleifera* is significant due to its ability to influence immune responses, particularly in relation to neuroinflammation (9), a key factor in the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's. In the central nervous system (CNS), microglia serve as the primary immune surveillance cells. Their role involves identifying pathological changes and initiating inflammatory responses (10). When stimulated by agents such as lipopolysaccharide (LPS), microglia shift from a resting state to an activated state, releasing pro-inflammatory mediators, including cytokines, reactive oxygen species (ROS), and nitric oxide (NO). While this response is crucial for host defence, chronic or uncontrolled activation can lead to prolonged inflammation and neuronal damage (11). Therefore, modulating microglial activity to promote a shift from the pro-inflammatory (M1) phenotype to the anti-inflammatory (M2) phenotype has emerged as a promising therapeutic strategy (12). *Moringa oleifera* contains bioactive compounds that affect pathways such as NF-κB and MAPKs, which suggests its potential as a modulator of microglial responses, offering neuroprotection by reducing excessive inflammation within the CNS.

Although *M. oleifera* has been extensively studied for its antioxidant and anti-inflammatory effects in many peripheral immune cells, its direct impact on microglial-driven inflammation remains underexplored. In particular, the role of *M. oleifera* leaf ethanol extract (MoETE) in modulating inflammatory responses in BV2 microglia has received limited scientific attention. Since microglia are crucial in central nervous system (CNS) immune responses (10), understanding how MoETE influences their behaviour is vital for assessing its therapeutic potential in neurological disorders.

Therefore, this study aims to examine the immunomodulatory effects of *Moringa oleifera* leaf ethanol extract on BV2 microglial cells, specifically investigating its impact on cell viability, nitric oxide production, and apoptotic response under both resting and LPS-stimulated conditions. This research addresses a gap in current knowledge and provides a scientific basis for the therapeutic development of *M. oleifera* in the treatment of inflammation-related neurodegenerative disorders.

## MATERIALS AND METHODS

### Source of *M. oleifera* leaves

The leaves of *M. oleifera* were obtained from a sole supplier in Bangsar, Selangor. The collected leaves underwent a washing process involving two rinses with distilled water, followed by oven-drying at 40°C for 72 hours. Subsequently, the dried leaves were ground into a powder, weighed, and then assessed for microbial content, heavy metals, and purity. The resultant powder was stored in a chiller at 4°C until required for use.

### *M. oleifera* extraction and storage

The leaves of *M. oleifera* underwent ethanol extraction at the Phytochemistry Unit, Herbal Medicine Research Centre (HMRC) of the Institute of Medical Research (IMR) in Kuala Lumpur. Using sonication extraction, the dry powdered *M. oleifera* leaves were extracted with 70% ethanol at a 50 g to 500 mL ratio and left at room temperature for 1 hour. The extract was filtered using filter paper and then concentrated under pressure by a rotary evaporator (Buchi Rotavapor R210/215, Switzerland) at 40°C to obtain a crude extract. The concentrated extracts underwent freeze-drying to yield a dry test material, which was stored at -20°C until further use.

### Preparation of *M. oleifera* stock solution

The stock solution of the *M. oleifera* ethanol extract (MoETE) was prepared by dissolving 100 mg of extract in 1 mL of filtered dimethyl sulfoxide (DMSO). From this stock, serial dilutions were performed in complete DMEM media to generate working concentrations of 0.1, 1.0, 10.0, 100.0, 200.0, 500.0 and 1000.0 µg/mL. Fresh stock solutions were prepared before the initiation of every new assay.

### Cells and cell culture conditions

The microglial BV2 cell line was obtained from the Microglia Research Group, Immunology Laboratory, Faculty of Medicine and Health Sciences (FMHS). The cells were grown in the DMEM (Gibco, Thermo Fisher Scientific, United States) medium consisting of 5% fetal bovine serum (FBS), 1% penicillin and streptomycin, 0.5% amphotericin, 0.1% gentamicin, 0.1% insulin, sodium bicarbonate, and non-essential amino acid (NEAA) before incubation at 37°C for 24 hours under a humidified atmosphere of 5% CO2. Cells were then subcultured once they reached confluency between 80% to 90%. This involves detaching the cells using 0.25x trypsin-EDTA, followed by neutralisation with media, and then reseeding in a fresh medium to maintain optimal growth conditions.

### MTT Assay

The3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium

bromide (MTT) assay was utilised to determine the viability of the cells. Briefly, the BV2 cells were seeded at 1.5 x $10^4$ cells into the wells of a 96-well plate before a 24-hour treatment at 37°C with MoETE at different concentrations (0.1-1000 µg/mL) for the experimental group, and with only complete media for the blank control group for 24 hours. The media in each well was removed and replaced with 100 µl of 0.5 mg/mL MTT reagent before 4 hours of incubation. The MTT solution was then removed, and upon addition of 100 µl of dimethyl sulfoxide (DMSO) per well, the mitochondrial succinate dehydrogenase in the living cells reduced from yellow to water-insoluble blue-purple crystals (formazan crystals), followed by 5 minutes of incubation. The absorbance was measured using an automated ELISA microplate reader (BIOBASE, China) at a wavelength of 570 nm.

### Griess Assay

The Griess assay was used to measure the production of nitric oxide (NO) in the cell supernatants, following the protocol outlined in the Griess Reagent System kit (Promega, United States). A standard curve was made by serial dilution with concentrations ranging from 1.56 to 100 µM of sodium nitrite (NaNO2). In a 96-well plate, 50 µL of cell culture supernatant was mixed with an equal volume of NaNO2. After 10 minutes of incubation in the dark, the absorbance was measured at 540 nm using an automated ELISA (BIOBASE, China) microplate reader, and the concentration of NO was quantified based on the NaNO2 standard curve.

### Apoptosis Assay

The apoptosis assay was performed to determine the extent of programmed cell death. The BV2 cells were seeded in 6-well plates in complete DMEM media. The cells were then treated with MoETE at concentrations of 0.1 and 1000.0 µg/mL. The plates were incubated for 24 hours before harvesting, and then washed twice with cold, sterile PBS to ensure that all cells were collected. The cells were then incubated with 100 µL of 10x Stain Buffer (BSA), followed by staining with 5 µL of Annexin V and 5 µL of PI in the treated cells. The stained cells were then incubated for 15 minutes in the dark, followed by resuspension in 400 µL diluted BSA. Acquisition cells were then obtained using flow cytometry (BD FACSCantoTM, United States), and data analysis was performed using software (BD FACSDiva, United States). Approximately a minimum of 10,000 cells were analysed from each sample. The cellular fraction that is negative for both annexin and propidium iodide is generally regarded as non-apoptotic. Conversely, the annexin V-FITC$^+$/PI$^-$ population is categorised as early apoptotic cells, and the annexin+/PI+ population as late apoptotic cells. Cells that are annexin V-/PI+ are considered disintegrated cells that have lost their plasma membrane.

### Statistical analysis

All data values were reported as mean (±) standard deviation (SD). One-way ANOVA was performed to compare all pairs to the blank control groups, followed by a post-hoc Dunnett's test. Two-way ANOVA for pairwise comparison, followed by Sidak's. Statistical significance was set at $p \leq 0.05$. All data were analysed using GraphPad Prism Version 10.0 (GraphPad, United States).

## RESULTS

### Cytotoxicity Analysis

Fig. 1 shows that MoETE treatment at concentrations ranging from 0.1 to 500.0 µg/mL did not significantly reduce BV2 cell viability relative to the untreated control (where 0.0 µg/mL: untreated control group), indicating that these concentrations were considered non-cytotoxic. A modest increase in viability was observed at 200.0 and 500.0 µg/mL. However, a significant reduction in viability was detected at 1000.0 µg/mL, suggesting cytotoxicity at this highest concentration.

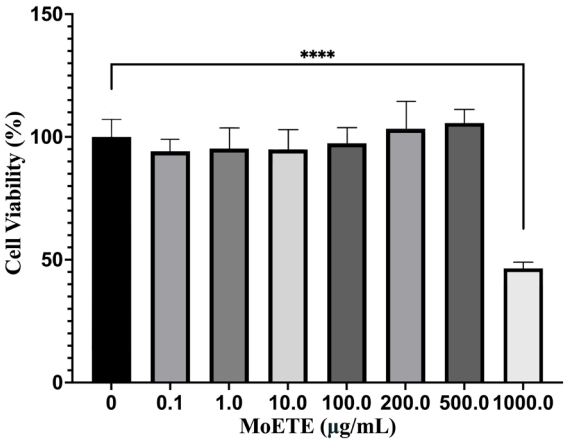


**Fig. 1: Effect of MoETE at different concentrations on BV2 cell viability. The control group (0.0 µg/ml) demonstrated 100.00 +/- 0.02 cell viability, establishing the baseline for comparison. Data are presented as the mean ± SD values of three independent experiments conducted in triplicate. Comparison between different groups was performed with one-way ANOVA, followed by post-hoc Dunnett's test. **** $p < 0.0001$ represents a significant difference compared to the untreated control group.**

### Quantification of Nitrite

### Standard Curve and Regression Analysis

The standard curve of the Griess assay (Fig. 2) was generated using sodium nitrite ($NaNO_2$) at concentrations ranging from 0 to 100 µM.

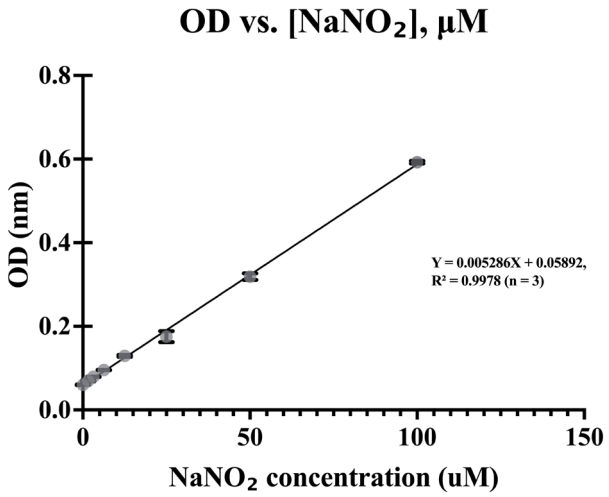


**Fig. 2: Scatter plot of standard curve with best-fit line and regression equation.**

The optical density (OD) values increased proportionally with $NaNO_2$ concentration, and the background-corrected OD values indicated a clear linear relationship. Simple linear regression analysis yielded the following best-fit equation:

$$Y = 0.005286X + 0.05892$$

where Y is the absorbance and X is the nitrite concentration in µM. The regression analysis demonstrated a good fit, with an R2 value of 0.9978, indicating that over 99% of the variance in absorbance is explained by nitrite concentration. The slope was significantly non-zero ($P<0.0001$), confirming the reliability of the standard curve for interpolating unknown concentrations.

### Nitrite Levels in Treatment Groups

#### Unstimulated BV2 cells

Fig. 3 shows that in unstimulated BV2 cells, MoETE treatment resulted in a stable nitrite production, ranging from approximately 10.240 ± 0.768 to 10.980 ± 0.353 µM in low-to-moderate concentrations. Significant increases in nitrite levels were observed at higher concentrations, particularly at 500.0 and 1000.0 µg/mL, indicating that higher concentrations of MoETE may enhance nitric oxide (NO) synthesis in resting BV2 microglial cells.

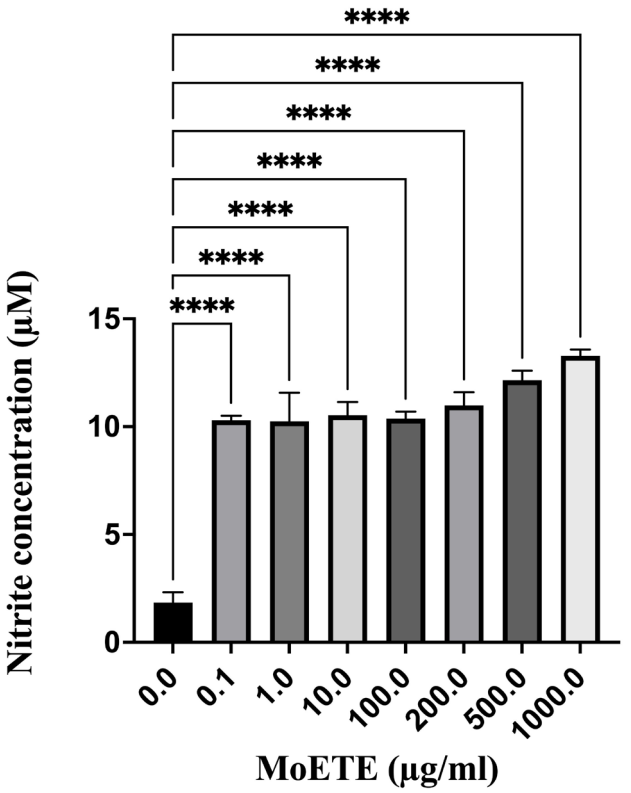


**Fig. 3: Effect of MoETE on nitrite production in unstimulated BV2 cells. Data represent mean ± SD of two independent experiments with triplicate. Comparisons among different concentrations were performed with one-way ANOVA, with post-hoc Dunnett's post-hoc test confirming that treatment concentration significantly affects nitrite levels. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ represents a significant difference.**

#### LPS-stimulated BV2 cells

Fig. 4 shows that in LPS-stimulated BV2 cells, MoETE modulated nitrite levels in a concentration-dependent manner, with concentrations ranging from 7.260 ± 0.333 to 10.510 ± 0.453 µM. While nitrite ranges were lower overall than in unstimulated conditions, treatment with the low-to-moderate concentrations of MoETE (< 500.0 µg/mL) significantly attenuated LPS-stimulated nitrite production, suggesting anti-inflammatory potential through suppression of nitric oxide (NO) production. Although a significant difference was observed relative to the untreated control group (0.0 µg/mL), where the highest concentration (1000.0 µg/mL) reached NO

levels almost to baseline (10.510 ± 0.453 µM). Nitrite concentrations (µM) were determined following 24-hour treatment with MoETE at concentrations ranging from 0.1 to 1000.0 µg/mL in the presence of LPS (1.0 µg/mL).

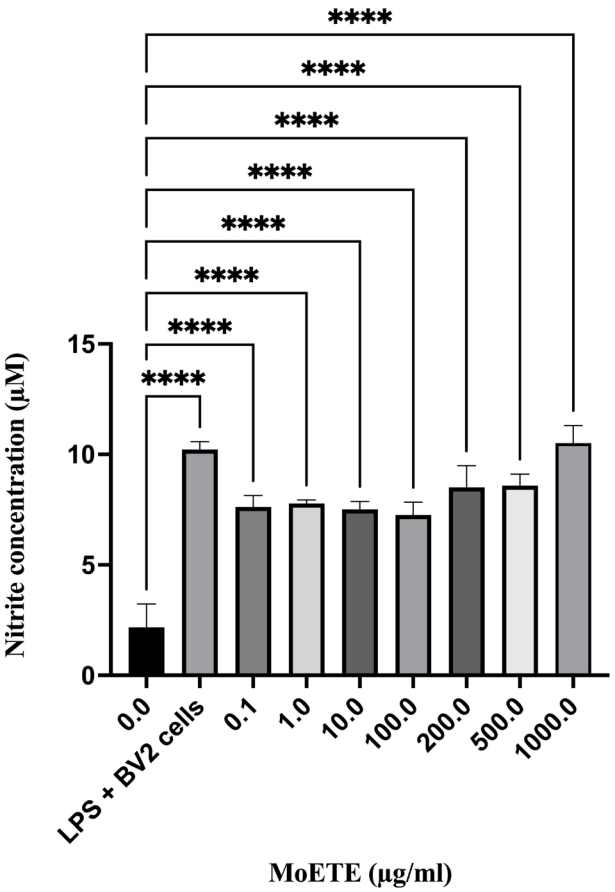


**Fig. 4: Effect of MoETE treatment on nitrite production in LPS-stimulated BV2 cells. Data represent the mean ± SD of two independent experiments with triplicate measurements. Comparisons among different groups were performed using one-way ANOVA, which indicated treatment effects on nitrite production under stimulation conditions, followed by a post-hoc Dunnett's test. **** p < 0.0001, *** p < 0.001, and * p < 0.05 represent a significant difference.**

## Comparison between unstimulated and LPS-stimulated conditions

Fig. 5 shows a comparison of nitrite production between unstimulated and LPS-stimulated conditions, revealing significant modulation by MoETE across all concentrations ($p < 0.05$–$0.0001$). It demonstrates that nitrite levels in both unstimulated cells and LPS-stimulated cells consistently produced those at low-to-moderate MoETE concentrations, with significant differences at nearly all concentrations (****p < 0.0001 to ***p < 0.001). However, at the highest MoETE concentration (1000.0 µg/mL), both conditions converge, producing comparable nitrite levels. MoETE produced stable nitric oxide production in unstimulated cells while concurrently attenuating LPS-stimulated nitric oxide elevations, highlighting its immunomodulatory effect depending on cellular activation status.

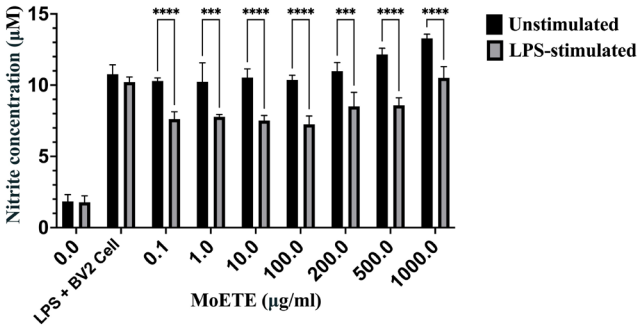


**Fig. 5: Effect of modulation of nitrite production by MoETE treatment between unstimulated and LPS-stimulated BV2 microglial cells. Comparisons among pairwise groups were performed with two-way ANOVA, followed by Sidak's multiple comparisons test was performed to assess significance. **** p < 0.0001, *** p < 0.001, ** p < 0.01, and * p < 0.05 represent a significant difference.**

## Analysis of Cell Viability and Apoptosis

Fig. 6A and 6B show that the unstained BV2 cell population exhibited minimal background fluorescence, with 100% of cells classified as viable. This confirms the specificity of the staining protocol and supports the validity of the gating parameters for subsequent analysis of apoptosis and necrosis. In untreated BV2 cells, viability remained high (90.3%), with minimal early (3.89%) and late (2.64%) apoptosis, and low necrosis (3.17%), reflecting the baseline physiological conditions in culture. These values serve as reference points for interpreting treatment-induced cytotoxic effects (Figure 6C). Fig. 6D illustrates that exposure of BV2 cells to 0.1 µg/mL MoETE resulted in a modest reduction in viability (78.1%) and an increase in early (11.1%) and late (9.92%) apoptosis, with minimal necrosis (0.84%). This suggests the presence of early signs of programmed cell death at low concentrations, without significant necrotic damage. In Fig. 6E, it is shown that at a concentration of 1000.0 µg/mL, MoETE significantly reduced cell viability to 70.1%. This was accompanied by a notable increase in late apoptosis (15.0%) and elevated necrosis (10.5%), indicating cytotoxicity at this high dosage. Early apoptosis was reduced to 4.49%, suggesting a progression towards later apoptotic stages and cell death.

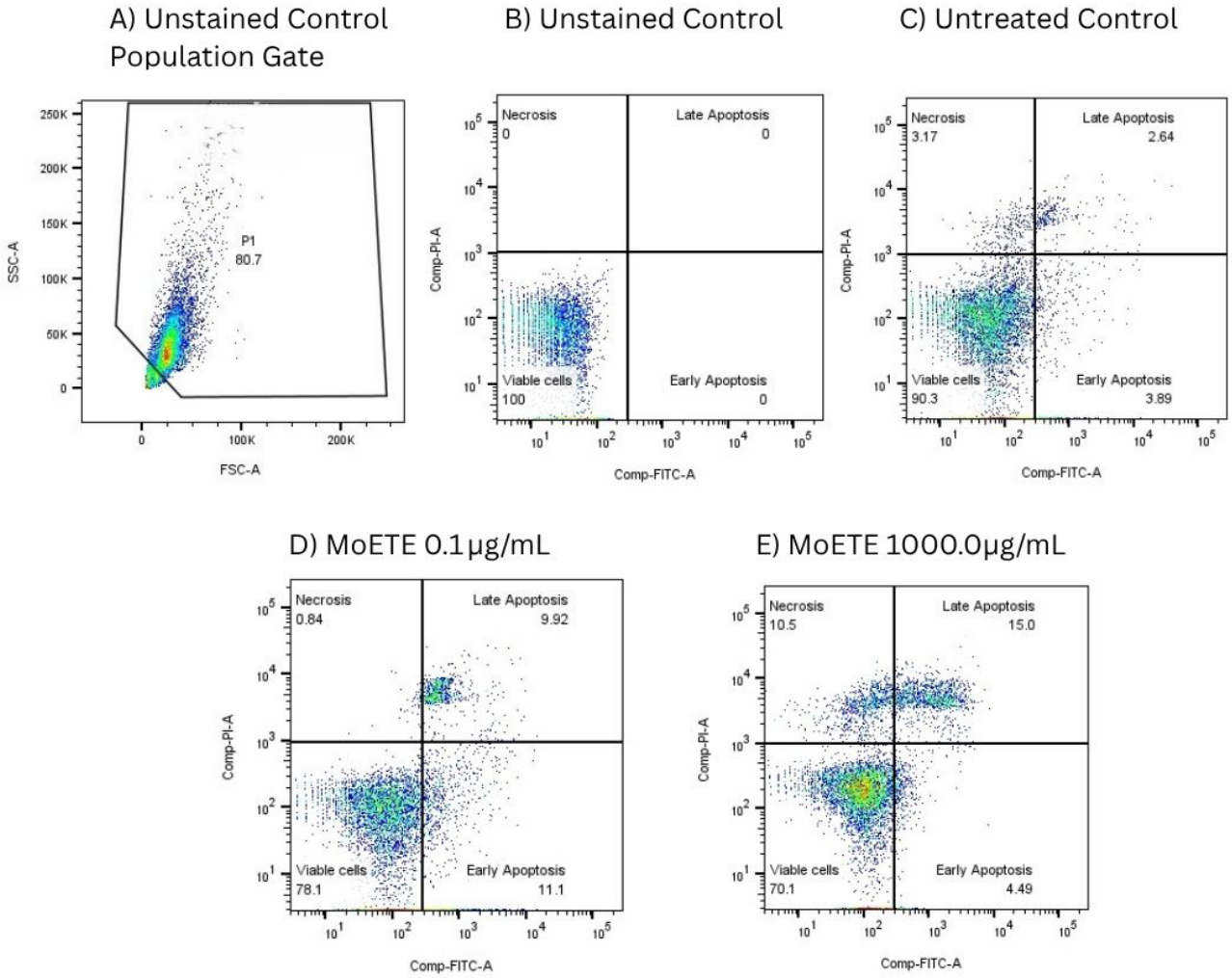


**Fig. 6: The MoETE supplementation at a higher dose induces apoptosis. Fig. 6A & B show the unstained BV2 cells with high viability. Fig 6C indicates the physiological level of apoptosis in BV2 cells. When BV2 cells are treated with 0.1 and 1000.0 µg/ml MoETE, the apoptosis level remains at the physiological level for the lower concentration, but the higher concentration induces apoptosis.**

## DISCUSSION

This study was conducted to examine the effects of *Moringa oleifera* leaf ethanol extracts (MoETE) on BV2 microglial cells, with a focus on its potential immunomodulatory properties by assessing cell viability, nitric oxide (NO) production, and cell-death profiling (annexin-V/PI flow cytometry). BV2 cells, recognised as a reliable model of resident brain macrophages, offer an in vitro framework for studying microglial activation, which is characterised by abnormal nitric oxide production — a hallmark of neuroinflammation linked to neurodegenerative disorders (13).

In accordance with previous studies on *M. oleifera's* abundant antioxidant and anti-inflammatory phytochemicals (14), low-to-moderate concentrations of MoETE (0.1–100.0 µg/mL) did not significantly affect BV2 cell viability, as shown in Table I. In other words, viability assays demonstrated approximately 90-105% viability relative to the untreated control group, consistent with previous findings (14, 23). A study by Sivaprakasam et al. reported similar results, where the viability of BV2 cells retained unaffected from low-to-moderate concentrations of Moringa leaf ethanol extract. Thus, these low to moderate MoETE concentrations exerted no measurable effect on cellular metabolic activity, signifying that MoETE is non-cytotoxic at these levels. In contrast, the highest concentration of MoETE tested (1000.0 µg/mL) caused a significant reduction in viability to 46.44% ($p < 0.0001$ vs. untreated control group), indicating cytotoxicity as supported by a previous study (15). This reaction indicates a concentration threshold beyond which the Moringa extract's load may disturb cellular redox balance; this redox "priming" triggers activation-induced apoptosis instead of necrosis in microglial cells (16). The observed cytotoxic effects at high concentrations may potentially be attributed to the pro-oxidant properties of certain Moringa compounds, particularly isothiocyanate, which are recognised to

show dual redox activity depending on dosage and cellular environment (17).

Interestingly, at a concentration of 0.1 µg/mL MoETE, the MTT assay demonstrated over 90% cell viability, as indicated in Table 1. In contrast, flow cytometry data revealed only 78.1% viable cells, as shown in Fig. 8. This difference likely results from the trypsinisation step required for the flow cytometry procedure. Prolonged exposure to trypsin can temporarily induce cellular stress and remove membrane proteins, resulting in the externalisation of phosphatidylserine on the cell surface (18–19). In fact, even a short extension of trypsin exposure may cause additional Annexin-V–positive "early apoptotic" events (19). Specifically, some BV2 cells retained metabolic activity (as indicated by the MTT assay) but exhibited Annexin positivity due to membrane disruption. Hence, extended trypsinisation at this concentration probably exaggerated the apoptotic cell count in the flow assay, explaining the slight discrepancy between the two viability assessments.

The apoptosis analysis further supports the dose-dependent effects of MoETE on BV2 cells. Flow cytometry showed that untreated control cells (0.0 µg/mL: BV2 cells without any treatment) had a high viability of 90.3%, with minimal apoptotic and necrotic features, indicating normal culture conditions. Exposure to 0.1 µg/mL MoETE resulted in a viability decline to 78.1%, with increased early (11.1%) and late apoptosis (9.92%), while necrosis remained low (0.84%). This small reduction may suggest a cellular stress response (20) rather than direct cytotoxicity, and as previously noted, could partly be due to technical factors such as prolonged trypsinisation during sample preparation. At the highest concentration (1000.0 µg/mL), cell viability dropped to 70.1%, with significant increases in late apoptosis (15.0%) and necrosis (10.5%), indicating substantial cytotoxic stress. These results support the MTT findings and confirm that high concentrations of MoETE likely induce programmed cell death due to disruption of cellular redox balance (16). The apoptotic data, combined with the metabolic and nitric oxide results, strengthen the understanding that MoETE presents a low-risk profile at lower doses but can be cytotoxic when used excessively.

In unstimulated BV2 cells, treatment with MoETE at concentrations ranging from 0.1 to 1000.0 µg/mL caused a concentration-dependent increase in nitrite levels. The increase at low-to-moderate concentrations may be due to redox-active phytochemicals such as quercetin, kaempferol, and chlorogenic acid, which are known to modulate redox signalling and increase low-level iNOS expression under non-inflammatory conditions (20–21), supporting physiological NO production without causing cytotoxicity. However, at higher concentrations, the further rise in nitrite levels is likely caused by redox imbalance and cellular stress, where excessive bioactive compounds, particularly isothiocyanates, may act as pro-oxidants, inducing stress-related nitric oxide (NO) release as a compensatory response to oxidative damage (22–23). These results reveal contrasting cellular responses across different concentration ranges, highlighting the importance of precise dosing when using MoETE as a microglial immunomodulatory agent. In LPS-stimulated BV2 cells, MoETE demonstrated an inhibitory effect on nitric oxide (NO) production, especially at low-to-moderate concentrations. As illustrated in Table III, nitrite levels were significantly reduced relative to the unstimulated condition, with measurements of 7.619 ± 0.296 µM (0.1 µg/mL), 7.512 ± 0.209 µM (10.0 µg/mL), and 7.260 ± 0.333 µM (100.0 µg/mL), indicating substantial inhibition of LPS-stimulated nitric oxide (NO) production. This suppression suggests that MoETE actively downregulates inflammatory responses, presumably by inhibiting the NF-κB/iNOS pathway, as documented for Moringa phytochemicals such as quercetin and isothiocyanates (17). At higher concentrations (200.0–1000.0 µg/mL), the inhibitory effect was attenuated, resulting in nitric oxide (NO) levels of 8.509 ± 0.568 µM, 8.584 ± 0.305 µM, and 10.510 ± 0.453 µM, respectively. Notably, at 1000 µg/mL MoETE, nitric oxide (NO) levels nearly approached those observed in the unstimulated condition (13.280 ± 0.171 µM), suggesting a reversal of the anti-inflammatory effect and potential induction of oxidative stress at high concentrations (14). This dose-dependent reversal, characterised by suppression at lower concentrations and a diminished effect at higher concentrations, underscores MoETE's immunomodulatory properties, which involve its ability to differentially regulate nitric oxide (NO) production based on the inflammatory state and dosage. This aligns with existing research on Moringa's phytochemicals, where flavonoids and isothiocyanates act as modulators of the NF-κB/iNOS axis, supporting its therapeutic potential. Future research will incorporate real-time NO measurement, additional inflammatory stimuli, and molecular pathway analysis to comprehensively understand MoETE's therapeutic potential in neuroinflammatory conditions.

## CONCLUSION

In conclusion, this study showed that *Moringa oleifera* ethanol leaf extract (MoETE) has dose-dependent effects on BV2 microglial cells. At low to moderate concentrations (0.1–100.0 µg/mL), MoETE maintained cell viability, did not induce apoptosis, and significantly reduced LPS-stimulated nitric oxide (NO) production, indicating a safe and immunosuppressive profile. Conversely, higher concentrations caused notable cytotoxicity, evidenced by decreased metabolic activity, increased apoptotic and necrotic cell populations, and elevated NO levels. These results suggest that MoETE possesses immunomodulatory properties by differentially regulating microglial activation and inflammatory

responses based on the cellular environment and dosage.

**ACKNOWLEDGEMENT**

This project is partially supported by the NKEA Research Grant Scheme (NRGS), "Regenerative and Immunomodulatory Activity of Standardised Ethanolic Extract of *Moringa oleifera* Lam. Leaves on Normal and Immunosuppressed Animal Models", Project Number: NH1015D072.

**REFERENCES**

1. Izah SC, Ogidi OI, Ogwu MC, Salimon SS, Yusuf ZM, Akram M, et al. Historical perspectives and overview of the value of herbal medicine. Reference Series in Phytochemistry. 2023;1:1–33. doi:10.1007/978-3-031-21973-3_1-1
2. Dashti MG, Khan MSS. Herbal remedies for lifestyle diseases: Managing and preventing diabetes, obesity, and cardiovascular conditions. Australian Herbal Insight. 2022;5(1):1–9. doi:10.25163/ahi.5121069
3. Rathore J, Das CR. *Moringa oleifera*. International Journal of Health Sciences. 2022;6(S1):6952–76. doi:10.53730/ijhs.v6ns1.6471
4. Prajapati C, Ankola M, Upadhyay TK, Sharangi AB, Alabdallah NM, Al-Saeed FA, et al. *Moringa oleifera*: Miracle plant with a plethora of medicinal, therapeutic, and economic importance. Horticulturae. 2022;8(6):492. doi:10.3390/horticulturae8060492
5. Ghosh C, Saha M, Chakraborty A, Ghosh P, Chatterjee S. *Moringa oleifera*: An edible medicinal plant. International Journal of Herbal Medicine. 2023;11(5):159–64. doi:10.22271/flora.2023.v11.i5b.902
6. Divya S, Pandey VK, Dixit R, Rustagi S, Suthar T, Atuahene D, et al. Exploring the phytochemical, pharmacological and nutritional properties of *Moringa oleifera*: A comprehensive review. Nutrients. 2024;16(19):3423. doi:10.3390/nu16193423
7. Abdull Razis AF, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera*. Asian Pacific Journal of Cancer Prevention. 2014;15(20):8571–6. doi:10.7314/apjcp.2014.15.20.8571
8. Chiș A, Noubissi PA, Pop OL, Mureșan CI, Fokam Tagne MA, Kamgang R, et al. Bioactive compounds in *Moringa oleifera*: Mechanisms of action, focus on their anti-inflammatory properties. Plants. 2024;13(1):20. doi:10.3390/plants13010020
9. Azlan UK, Khairul Annuar NA, Mediani A, Aizat WM, Damanhuri HA, Tong X, et al. An insight into the neuroprotective and anti-neuroinflammatory effects and mechanisms of *Moringa oleifera*. Frontiers in Pharmacology. 2023;13. doi:10.3389/fphar.2022.1035220
10. Pallarйs-Moratalla C, Bergers G. The ins and outs of microglial cells in brain health and disease. Frontiers in Immunology. 2024;15. doi:10.3389/fimmu.2024.1305087
11. Ahmad MA, Kareem O, Khushtar M, Akbar M, Haque MR, Iqubal A, et al. Neuroinflammation: A potential risk for dementia. International Journal of Molecular Sciences. 2022;23(2):616. doi:10.3390/ijms23020616
12. Zhao MM, Duan JY, Shen Y, Liu Y, Zeng KW. Recent advances in neuroinflammation prevention and therapy: The role of natural products and underlying mechanisms based on molecular targets. British Journal of Pharmacology. 2024; doi:10.1111/bph.16404
13. She Y, Shao C, Liu Y, Huang Y, Yang J, Wan H. Catalpol reduced LPS-induced BV2 immunoreactivity through NF-κB/NLRP3 pathways: An in vitro and in silico study. Frontiers in Pharmacology. 2024;15. doi:10.3389/fphar.2024.1415445
14. Arulselvan P, Choi DK, Sivaprakasam G, Ganesan P, Muniandy K, Park SY, et al. Attenuation of lipopolysaccharide-induced neuroinflammatory events in BV-2 microglial cells by *Moringa oleifera* leaf extract. Asian Pacific Journal of Tropical Biomedicine. 2019;9(3):109–9. doi:10.4103/2221-1691.254604
15. Adefegha SA, Assmann CE, Rosa M, Melazzo C, Emanuelli T. *Moringa oleifera* modulates cholinergic and purinergic enzymes activity in BV-2 microglial cells. Metabolic Brain Disease. 2021;36(4):627–38. doi:10.1007/s11011-020-00659-3
16. Sita G, Hrelia P, Tarozzi A, Morroni F. Isothiocyanates are promising compounds against oxidative stress, neuroinflammation and cell death that may benefit neurodegeneration in Parkinson's disease. International Journal of Molecular Sciences. 2016;17(9):1454. doi:10.3390/ijms17091454

17. Habtemariam S. Anti-inflammatory therapeutic mechanisms of isothiocyanates: Insights from sulforaphane. Biomedicines. 2024;12(6):1169–9. doi:10.3390/biomedicines12061169
18. Lordon B, Campion T, Gibot L, Gallot G. Impact of trypsin on cell cytoplasm during detachment of cells studied by terahertz sensing. Biophysical Journal. 2024;123(16):2476–83. doi:10.1016/j.bpj.2024.06.011
19. Yan G, Efferth T. Cell harvesting methods affect cellular integrity of adherent cells during apoptosis detection. Anticancer Research. 2018;38(12):6669–72. doi:10.21873/anticanres.13034
20. Obaid H, Alshebremi M, Babiker AY, Rahmani AH. The role of quercetin, a flavonoid in the management of pathogenesis through regulation of oxidative stress, inflammation, and biological activities. Biomolecules. 2025;15(1):151–1. doi:10.3390/biom15010151
21. Huang J, Xie M, He L, Song X, Cao T. Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. Frontiers in Pharmacology. 2023;14. doi:10.3389/fphar.2023.1218015
22. Mthiyane FT, Dludla PV, Ziqubu K, Mthembu SXH, Muvhulawa N, Hlengwa N, et al. Corrigendum: A review on the antidiabetic properties of *Moringa oleifera* extracts: Focusing on oxidative stress and inflammation as main therapeutic targets. Frontiers in Pharmacology. 2023;14. doi:10.3389/fphar.2023.1142410
23. Bakre A, Basar N, Velagapudi R, Sarker S, Ademowo O, Olajide O. *Moringa oleifera* inhibits neuroinflammation in LPS-activated BV2 microglia. The FASEB Journal. 2015;29(S1). doi:10.1096/fasebj.29.1_supplement.lb508