Buletin SK 10(2)(2026): 157-166
https://doi.org/10.17576/bsk-2026-10(2)-09 ISSN 2250-1852

Artikel Asli /Original Article

Cytotoxic Effects Induced by Methiopropamine (MPA) in BV2 Microglia

Kesan Sitotoksik Aruhan *Methiopropamine* (MPA) dalam Mikroglia BV2

AHMAD ROHI GHAZALI[1*],ADAM MUHAMMAD ZACKRY ZULKIFLY[1], NURUN NAJIBAH HAJI AZYRIN[2], SITI FATIMAH IBRAHIM[3], NORAZRINA AZMI[4]

[1]Centre for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia (Kampus Kuala Lumpur), Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

[2]Pengiran Anak Puteri Rashidah Sa'adatul Bolkiah (PAPRSB) Institute of Health Sciences, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong BE1410, Brunei Darussalam

[3]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Cheras 56000, Kuala Lumpur, Malaysia

[4]Centre for Drug and Herbal Development (CDHD), Faculty of Pharmacy, Universiti Kebangsaan Malaysia (UKM), 50300 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia

*Corresponding author; email: rohi@ukm.edu.my

Received date: 16 July 2026
Revised date: 22 October 2026
Accepted date: 23 October 2026
Published date: 26 October 2026

ABSTRACT

The global rise of new psychoactive substances (NPS) creates significant concerns for public health and drug regulation. Methiopropamine (MPA), a thiophene derivative of methamphetamine (MA), is a stimulant-type NPS that has emerged on cyber market in 2010. Despite reports of cardiovascular and neuropsychiatric toxicity, there is limited evidence of cellular toxicity in microglia, the brain's key immune cells involved in neuroinflammation and neurodegeneration. This work aims to evaluate the cytotoxic effects of MPA on BV2 microglial cells and estimate the IC_{50} values of MPA and menadione (positive control) using MTT assay. BV2 cells were treated with MPA and menadione at varying doses of 1, 0.5, 0.25, 0.125, 0.0625 mM for 24 hours. Cell viability was determined by mitochondrial metabolic activity. Results showed MPA induced a small decline in BV2 cell viability, with 66% viability at 1 mM where it was statistically significant compared to the untreated control ($p < 0.05$), which was still above the 50% threshold. Menadione showed a statistically significant dose-dependent drop with an estimated IC_{50} of 0.02 mM ($p < 0.05$), indicating the assay's sensitivity. These data show that MPA reduced cell viability significantly at the highest concentration tested (1mM) and that BV2 microglia might be able to withstand modest oxidative stress via internal antioxidant defense, whereas menadione was highly cytotoxic (estimated $IC_{50} = 0.02$ mM) and significantly reduced the cell viability. Future research should extend MPA doses above 1 mM to identify its IC_{50} value and characterize early oxidative or inflammatory reactions associated with MPA-induced neurotoxicity.

Keywords: BV2 Microglia, Cytotoxicity, Menadione, Methiopropamine (MPA), New Psychoactive Substances (NPS).

ABSTRAK

Peningkatan global bahan psikoaktif baharu (NPS) menimbulkan kebimbangan terhadap kesihatan awam dan kawal selia dadah. Methiopropamine (MPA), iaitu terbitan *thiophene* bagi methamphetamine (MA), merupakan NPS jenis perangsang yang muncul di pasaran siber pada tahun 2010. Meskipun terdapat laporan mengenai ketoksikan kardiovaskular dan neuropsikiatrik, bukti mengenai ketoksikan selular terhadap mikroglia, iaitu sel imun utama otak yang terlibat dalam neuroinflamasi dan neurodegenerasi adalah terhad. Kajian ini bertujuan untuk menilai kesan sitotoksik MPA terhadap sel mikroglia BV2 serta menganggar nilai IC_{50} bagi MPA dan

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menadion (kawalan positif) menggunakan asai MTT. Sel BV2 diaruh dengan MPA dan menadion pada pelbagai dos iaitu 1, 0.5, 0.25, 0.125, dan 0.0625 mM selama 24 jam. Viabiliti sel ditentukan melalui aktiviti metabolik mitokondria. Hasil kajian menunjukkan MPA menyebabkan sedikit penurunan dalam viabiliti sel BV2, dengan viabiliti 66% pada kepekatan 1 mM. Penurunan ini adalah signifikan secara statistik berbanding kawalan yang tidak dirawat ($p < 0.05$), namun masih melebihi ambang 50%. Menadione menunjukkan penurunan yang signifikan secara statistik dan bergantung kepada dos dengan anggaran nilai IC_{50} sebanyak 0.02 mM ($p < 0.05$), yang membuktikan sensitiviti asai tersebut. Data ini menunjukkan bahawa MPA menyebabkan penurunan viabiliti sel secara signifikan pada dos tertinggi ujian (1mM) dan sel mikroglia BV2 mungkin berupaya menahan tekanan oksidatif sederhana melalui mekanisme pertahanan antioksidan dalaman. Sebaliknya, menadione didapati sangat sitotoksik (anggaran $IC_{50} = 0.02$ mM) dan mengurangkan daya hidup sel secara ketara. Kajian masa hadapan perlu melibatkan dos MPA melebihi 1 mM bagi mengenal pasti nilai IC_{50} serta mencirikan tindak balas awal oksidatif atau inflamasi yang dikaitkan dengan neurotoksisiti akibat MPA.

Kata Kunci: Bahan Psikoaktif Baharu (NPS), Mikroglia BV2, Menadion, *Methiopropamine* (MPA), Sitotoksisiti

INTRODUCTION

The global emergence of new psychoactive substances (NPS) has presented significant challenges to public health systems and regulatory authorities worldwide. They are compounds that are created to replicate the effects of conventional illegal drugs while avoiding legal constraints, and are often sold as “bath salts”, “designer drugs”, or “legal highs” (Bade et al. 2023; Bunaim et al. 2024). NPS falls into different categories based on their pharmacological effects, including cannabinoids, hallucinogens, depressants, and stimulants (Shafi et al. 2020; Tracy, Wood & Baumeister 2017). Among the various categories, stimulant-type substances represent the most frequently monitored class by both the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNODC) (Bunaim et al. 2024; Miliano et al. 2016).

Methiopropamine (MPA), chemically known as (1-thiophen-2-yl)-2 methylaminopropane), is a MA analogue designed to circumvent existing drug legislation (Bunaim et al. 2024). It is a synthetic stimulant known by various street names including “quicksilver” and legal cocaine or “synthacaine”, and was known to firstly appear on European online markets in 2010 as an alleged “legal high” (Cumba et al. 2015; European Monitoring Centre for Drugs and Drug Addiction & Europol 2013). The compound holds a structure similar to MA, except that the benzene ring is replaced with a thiopene ring (Figure 1) (Tuv et al. 2021).

MPA predominantly affects dopaminergic and noradrenergic neurotransmission through transporter inhibition, creating a pharmacological profile that is remarkably similar to that of conventional amphetamine-type stimulants (ATS) (Iversen et al. 2013; Rudin, Liechti & Luethi 2021). In vitro investigations have shown that MPA inhibits dopamine and norepinephrine transporters more potently than the serotonin transporter, resulting in higher synaptic concentrations of these monoamines (Iversen et al. 2013). The mechanism of action is responsible for the compound’s stimulant effect and contributes to its abuse potential, as demonstrated by locomotor sensitisation studies in rodent models and user complaints of dependence-like symptoms (Bunaim et al. 2024).

Apart from that, case reports described that MPA toxicity presents clinically as a wide range of side effects, including cardiovascular issues such as tachycardia and hypertension, as well as neuropsychiatric symptoms including agitation, paranoid ideations, and visual hallucinations. Other symptoms include psychosis, anxiety, nausea and vomiting (Daveluy et al. 2016; Deslandes et al. 2017; Lee et al. 2014; White et al. 2019). Preclinical studies in mice have shown considerable toxicity profiles, with chronic dosing in mice contributing to myocardial damage, renal injury, and mortality rates approaching 40% at higher dosages (30 mg/kg) (De-Giorgio et al. 2020). According to Bunaim et al. (2024), the proposed mechanism for MPA-induced neurotoxicity is dopaminergic and noradrenergic dysfunction and sympathetic pathway activation, which results in oxidative stress, microglial activation and neuroinflammation.

Despite the known toxicity and growing popularity of MPA usage, considerable knowledge gaps remain about its cellular causes of harm. The brain’s distinctive sensitivity to substance-induced harm, due to its high metabolic activity and low regenerating capacity, justifies research on MPA’s neurotoxicity (Trofin et al. 2025). The brain's reliance on oxidative metabolism also makes it particularly susceptible to Reactive Oxygen Species (ROS) and oxidative stress induced by MPA (Nguyen et al. 2019). Unlike other tissues, the brain has a low capacity for neuronal regeneration, meaning damage caused by MPA may result in long-term or irreversible deficits (Bunaim et al. 2026; Nguyen et al. 2019). MPA's neurotoxicity stems from its ability to disrupt dopaminergic and noradrenergic signalling, induce oxidative stress and neuroinflammation. These effects, combined with the brain's inherent vulnerability, underscore the importance of further research into MPA's cellular mechanisms of harm.

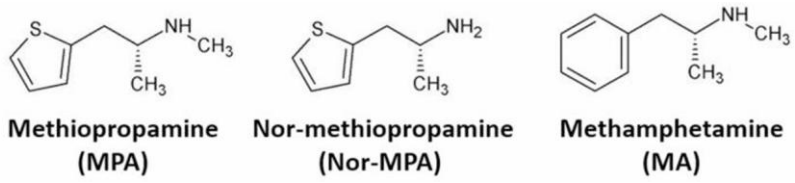


FIGURE 1 Structural formula of MPA, Nor-MPA, and MA. Figure taken from (Tuv et al. 2021).

Microglia, the brain's resident immune cells and main sensors of neuronal disturbance, play critical roles in neuroinflammation and neurotoxicity after exposure to psychoactive drugs (Li, Watkins & Wang 2024). In response to toxic assaults, these cells shift morphologically and functionally, changing from a damaged monitoring state to activated phenotypes with altered cytokine production and phagocytic activity. When they are chronically activated, the cells will persist its neuroinflammatory state and may implicate various brain diseases including Alzheimer's disease, Parkinson's disease, and depression (Audrain et al. 2021; He et al. 2022; Klawonn et al. 2021; Li, Watkins & Wang 2024; Yun et al. 2018; Zhao et al. 2021b). In addition, mitochondria plays a crucial role in maintaining microglia's normal function and integrity through regulation of cell metabolism and viability (Bader & Winklhofer 2020). According to Zhao et al. (2021a), this organelle proved that it plays an important part in innate and adaptive immune responses coordination, in which it links neurodegeneration and neuroinflammatory responses. Its dysfunction and the resulting ROS from the leaked electrons play a key role in the activation of microglia, both initially and continuously. Elevated ROS in microglia activates inflammatory and cell death pathways (Agrawal & Jha 2020; Chiurazzi et al. 2020; Regen et al. 2017; Rojo et al. 2014).

The BV2 microglial cell line has emerged as a critical in vitro model for studying neuroinflammation and neurotoxicity processes (Henn 2009). These immortalised murine microglial cells retain various properties of primary microglia, such as inflammatory response and the ability to generate cytokines and reactive oxygen species (ROS) (Stansley, Post & Hensley 2012). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay is a valid method for determining cell viability by measuring mitochondrial activity, as MTT reduction to formazan represent the reductive potential of living cells (Jian et al. 2019).

Current research on psychostimulant-induced microglial activation has mostly focused on traditional drugs such as MA and cocaine, with limited exploration into NPS such as MPA. Studies on methamphetamine's effects on microglia have revealed dose dependent activation patterns and neuroinflammatory responses, particularly in striatal areas (Hall et al. 2023; Loftis & Janowsky 2014). However, the specific cytotoxic effects of MPA on microglial cells are mostly unknown, indicating a crucial gap in the understanding of the compound's neurotoxic mechanisms. The primary aim of this study was to explore the cytotoxic effects of MPA on BV2 microglial cells. This study aimed to obtain the IC_{50} values of MPA.

MATERIALS AND METHODS

CELL LINES AND CULTURE CONDITION

The BV2 cells were kindly provided by Dr. Mohd Kaisan bin Mahadi (Faculty of Pharmacy, Universiti Kebangsaan Malaysia). The cells were purchased from the American Type Culture Collection (ATCC). The BV2 cells is an adherent type of cell grown in high glucose Dulbecco's Modified Eagle Medium (DMEM) (Capricorn Scientific, Germany) supplemented with 10% Fetal Bovine Serum (FBS) (Capricorn Scientific, Germany) and 1% penicillin-streptomycin (Capricorn Scientific, Germany). The culture media was made by combining 89% DMEM, 10% FBS, and 1% penicillin-streptomycin and kept at 4 °C. BV2 cells were grown initially at T-25 flask, then subculture into T-75 culture flasks at 37°C in a humid incubator with 5% CO_2. Before each subculture, all reagents (DMEM, PBS, and trypsin-EDTA) were heated to 37 °C. The flasks were rinsed with PBS once to remove any remaining serum, and then trypsin-EDTA (0.25%) was added to detach adherent cells. Following 5 minutes of incubation, the reaction was neutralised with complete medium, and the cell suspension was centrifuged at 1200 rpm for 5 minutes. The supernatant was removed, and the cell pellet was resuspended in new DMEM. Cell viability and density were measured using trypan blue exclusion and a haemocytometer. Cells were sub cultured until they reached approximately 90% confluence. Cells are used for tests when the cells' passage becomes passage 3-10 (maximum).

MPA STOCK PREPARATION

To make a 100 mM stock solution of MPA, 0.15526 g of MPA was dissolved in 10 mL of DMSO and filtered through a 0.22 µm filter. Serial dilutions in DMEM were performed to produce final concentrations of 1.0, 0.5, 0.25, 0.125, and 0.0625 mM. To prevent degradation, all preparations were

done in low-light conditions and stored at -20°C in aluminium-wrapped tubes.

MENADIONE STOCK PREPARATION

Menadione was used as a positive control for oxidative stress related cytotoxicity. A 100 mM menadione stock solution in DMSO was sterilized using a 0.22 µm syringe filter. Working doses of 1.0, 0.5, 0.25, 0.125, and 0.0625 mM were achieved with serial dilutions in full DMEM. Due to the compound's photosensitivity, all solutions were also made under low-light settings and stored at -20°C when not in use aluminium-wrapped tubes.

MTT CYTOTOXICITY ASSAY

The cytotoxicity of MPA in BV2 cells were evaluated through the MTT cell viability assay according to the method of Mosmann et al. (1983) with a few modifications. BV2 cells were seeded in 96-well plates with a density of 1×10^5 cells/mL (200 µL per well) and incubated overnight for attachment (Piper et al. 2022). After 24 hours, the medium was replaced with 200 µL of MPA-containing media at each concentration. Untreated cells served as the negative control, with blank wells containing only media. After 24 hours of exposure, 20 µL of MTT solution (5 mg/mL in PBS) was applied to each well and incubated for 4 h at 37°C. To dissolve the formazan crystals, the medium was aspirated and replaced with 200 µL of DMSO. Plates were gently shaken on a belly dancer for 15 minutes before being measured at 570 nm with a microplate reader (Thermo Fisher Scientific, US). Cell viability was determined as a percentage of absorbance compared to untreated control wells. Similar protocol was employed for menadione. Cell viability can be calculated by the formula below:

$$Cell\ Viability\ (\%) = \frac{Mean\ OD\ of\ treated\ cells}{Mean\ OD\ of\ negative\ control} \times 100\%$$

DATA ANALYSIS

All studies were carried out in six independent trials. Data were presented as mean ± standard error of mean (SEM). Statistical analysis was carried out using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Multiple groups were compared using one-way analysis of variance (ANOVA), with pairwise comparisons using Tukey's post-hoc test. Statistical significance was determined at $p < 0.05$. Menadione and MPA cytotoxic effects on BV2 cell viability were visualised using dose-response curves.

RESULTS

EFFECTS OF DIFFERENT CONCENTRATIONS OF MENADIONE ON BV2 CELL VIABILITY

As shown in Figure 2, BV2 microglial cells treated with increasing concentrations of menadione from 0.0625 to 1 mM significantly reduces cell viability. Menadione as the positive control to establish cytotoxicity showed an estimated IC_{50} of 0.02 mM. This value was determined by nonlinear regression using a four-parameter logistic model. However, this estimate was below the lowest concentration tested (0.0625 mM) and should therefore be interpreted cautiously as an extrapolated value. Compared with the untreated control (0 mM; 100% viability), even the lowest concentration of 0.0625 mM caused a significant decrease of approximately 97% which indicates severe cytotoxicity. Higher concentrations of 0.125 to 1 mM maintained similarly low viability values, proving that menadione exerts strong toxicity across all doses tested. Statistical analysis using one-way ANOVA revealed a highly significant difference among all treatment groups ($p < 0.05$). Tukey's post-hoc test further confirmed that all treated groups differed significantly from the untreated control, where $p < 0.05$. Results are expressed as the means ± SEM

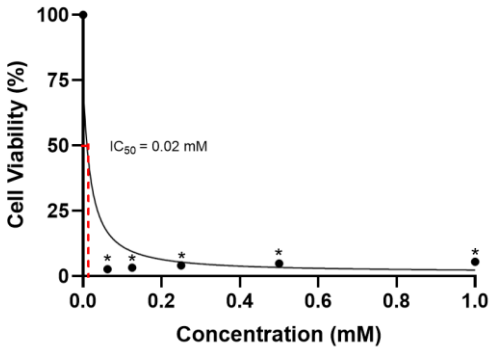


FIGURE 2 Cell viability of BV2 microglia treated with menadione control (0.0625 - 1 mM). Values are expressed as mean ± SEM (n=6). Statistical analysis by one-way ANOVA with Tukey's post-hoc test. Significant differences denote by * ($P < 0.0001$) compared with untreated control (0 mM)

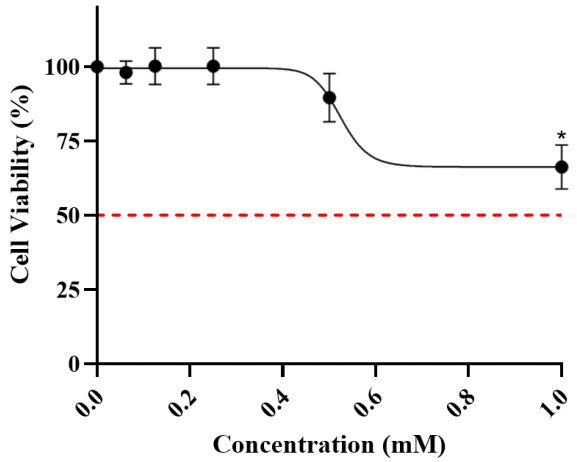


FIGURE 3 Cell viability of BV2 microglia treated with MPA (0.0625 - 1 mM).
Values are expressed as mean ± SEM (n=6). Statistical analysis by one-way ANOVA with Tukey's post-hoc test. Significant differences denote by * $p < 0.01$ compared with untreated control (0 mM).

TABLE 1 Menadione-induced cytotoxicity across various cell lines.

Cell Type	Species	IC_{50} (Mm)	Exposure	Reference
H4IIE hepatoma cell line	Rat	0.025	24 h	(Oztopcu-Vatan et al. 2015)
SK-N-MC neuroblastoma	Human (brain tumor)	0.0109	24 h	(Parandavar & Yazdanparast 2017)
SAS oral carcinoma	Human (squamous carcinoma)	0.0085	24 h	(Suresh, Raghu & Karunagaran 2013)
HEK293 embryonic kidney	Human (non-tumor)	0.0985	24 h	(Suresh, Raghu & Karunagaran 2013)
HaCaT keratinocyte	Human (non-tumor skin)	0.0745	24 h	(Suresh, Raghu & Karunagaran 2013)
Pancreatic carcinoma cells	Human (pancreatic cancer)	0.042	24 h	(Osada et al. 2008)

EFFECT OF DIFFERENT CONCENTRATIONS OF MPA ON BV2 CELL VIABILITY

According to the results of the cell viability in Figure 3, MPA reduced cell viability at higher concentration, although the IC_{50} value was not reached within the tested concentration range. MPA produced mild decrease in cell viability. At low concentrations of 0.0625 to 0.25 mM, cell viability remained constant between 98% to 100%. A modest decline occurred at 0.5 mM (89.56% + 8.103), followed by a more evident decrease to 66.25% + 7.381 at 1 mM. One-way ANOVA revealed significant differences among treated groups, where $p = 0.0016$, and Tukey's post hoc test identified 1 mM as the only concentration significantly different from the untreated control (adjusted $p = 0.0044$), while other concentrations were not significant. Despite this reduction, cell viability at 1 mM remained above 50% toxicity threshold, suggesting non-cytotoxic effects under the tested conditions. Results are expressed as the means ± SEM

DISCUSSION

The present study used the MTT assay to investigate the cytotoxic effects of MPA, a thiophene analogue of MA, on BV2 microglial cells. The results demonstrated that MPA exposure ranging from 0.0625 mM to 1 mM was not cytotoxic towards BV2 cells, as cell viability remained above the 50% threshold required to calculate an IC_{50} value. It is noted that from 0.0625 to 0.25 mM, the viability remained at approximately 100%, suggesting that MPA at these range of concentrations have no effect on BV2 cells. However, at 0.5 mM, the cell viability decreased to 89.56% and at the highest concentration of 1 mM, the observed viability reduction to 66%. These findings indicate a reduction in cell viability or metabolic activity, although the underlying mechanism, including possible mitochondrial stress, was not directly

investigated in this study. Although MPA is an NPS with documented clinical toxicity, its neurotoxic effects have not been thoroughly examined in vitro (Tuv et al. 2021). The IC_{50} is expected to be more than 1 mM of MPA. The current findings are the first direct evidence that MPA can induce microglial cell death at high concentrations, although further studies are needed to confirm whether this reduction involves cell death.

Notably, the cytotoxicity threshold for MPA appears to be quite high as lower doses had no significant effect on viability. This effect shows that under moderate exposure conditions, MPA may stimulate or stress microglia without killing them, whereas higher levels result in cell death. In vitro toxicological studies of stimulant NPS analogues to MPA, including synthetic cathinones and substituted amphetamines, show relatively modest acute cytotoxicity in brain-derived cell models, but there is substantial evidence of oxidative stress and neuroinflammatory effects at high concentrations. 3,4 Methylenedioxymethamphetamine (MDMA), a structural amphetamine analogue, had an IC_{50} of 243.6 μg/ml (approximately 1 mM) for 24 hours cytotoxicity in murine BV2 microglial cultures. It also elicited dose-dependent release of tumour necrosis factor-α (TNF- α), indicating microglial activation (Mohamad et al. 2022). Synthetic cathinones follow similar patterns. Mephedrone (4-MMC) did not significantly reduce viability in human neuroblastoma (SH-SY5Y) or astrocytoma (U-373) cell lines at sub-millimolar levels. In fact, 48 hours exposure to 100 μM mephedrone slightly increase MTT metabolic activity in these cells, and no measurable lactate dehydrogenase (LDH) release occurred even after 6 days at this concentration (Alanazi et al. 2024). Mephedrone's cytotoxic effects were observed at higher doses (≥500 μM), which is consistent with previous observations that β-keto amphetamines cause cell death only at high micromolar ranges and above (Alanazi et al. 2024; Den Hollander et al. 2014).

Other than that, MDPV, a methylenedioxypyrovalerone stimulant, reduced neuronal cell metabolism by approximately 15% at 300 μM after 24 hours. However, there was no loss of membrane integrity or significant LDH leakage at these concentrations. Many cathinone analogues require prolonged exposure of more than 24 hours and concentrations on the order of ≥1 mM to significantly impair cell viability. Reduction in cell viability is commonly observed only at millimolar levels across various drugs of abuse including cathinones, amphetamine-like stimulants, and phenethylamines (Sogos et al. 2021). The relatively high BV2 cell viability at 1 mM MPA may indicate a limited effect on cell viability under the tested conditions. However, oxidative stress was not directly measured in this study. Its neurotoxic potential is thought to derive from monoaminergic dysregulation and dopamine auto-oxidation, both of which indirectly produce ROS (Bunaim et al. 2024). However, Li et al. (2022) reported that BV2 microglia upregulated antioxidants such as heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) as part of an NRF2-mediated response under oxidative stress. This response may represent a possible explanation for the relatively small reduction in MPA-treated cell viability observed in the present study, although the involvement of endogenous antioxidant defenses remains to be investigated. In addition, our study used menadione as the positive control to compare with MPA. This is the first study to report the estimated IC_{50} of menadione in BV2 cells. Known as a strong ROS generator, menadione exhibited a strong dose-dependent decline in cell viability, with an estimated IC_{50} value of 0.02 mM in this study (Diaz De Barboza et al. 2017). The number is consistent with documented IC_{50} values for menadione in different cell lines assessed through MTT assay, where the values lie in between 0.0085 to 0.0985 mM, as shown in Table 1. Prior investigations have found IC_{50} values of 0.025 mM in H4IIE rat hepatoma cells and 0.042 mM in human pancreatic cancer cells (Osada et al. 2008; Oztopcu-Vatan et al. 2015). However, particularly sensitive cell lines such as the SAS human oral carcinoma have IC_{50} as low as 0.0085 mM (Suresh, Raghu & Karunagaran 2013). In the setting, the IC_{50} in BV2 cells is moderate, meaning it is higher than the most sensitive tumour cells but lower than those in certain non-transformed cells. BV2 microglia are less sensitive to menadione than SAS oral squamous carcinoma or SK-N-MC neuroblastoma cells, which have extremely low IC_{50} values of 0.0088 and 0.0109 mM respectively, indicating greater susceptibility. The BV2 IC_{50} is significantly lower than that of immortalised non tumorigenic human cell lines like HEK293 (embryonic kidney) and HaCaT (keratinocyte), which have IC_{50} values of 0.0985 mM and 0.0745 mM, respectively (Suresh, Raghu & Karunagaran 2013). In other words, the quantity of menadione necessary to kill 50% of the BV2 cells is comparable to that of many cancer cells, but substantially lower than the dose tolerated by non-tumour cells. Hence, microglial cells are more on the sensitive end of the range when compared to other cell types, although they are not the most sensitive of the cells studied.

The significant cytotoxicity of menadione in BV2 cells is consistent with its known mechanism of oxidative damage through superoxide overproduction and mitochondrial permeability transition (Diaz De Barboza et al. 2017). Even at 0.0625 mM, menadione induced reduction in cell viability, validating the assay's responsiveness and providing a baseline for comparing MPA's reduced effect. The significant difference in IC_{50} values illustrates the chemical and mechanistic distinctions

between MPA, which is a stimulant-type NPS, and conventional ROS inducers like menadione.

While present studies indicate that MPA is not cytotoxic up to 1 mM. The gradual reduction in cell viability observed with increasing MPA concentrations may indicate changes in cellular metabolic activity. However, the possibility of sublethal mitochondrial stress remains speculative because mitochondrial function was not directly assessed in this study. More research is needed to accurately determine the IC_{50} value utilising wider concentration ranges. Furthermore, studies focusing on oxidative stress indicators, mitochondrial membrane potential, and cytokine release could aid in understanding non-lethal functional consequences that occur prior to apparent cell death. Understanding the cytotoxic threshold of MPA is crucial from a toxicological and public health standpoint, as this drug is still commercially available online and is not subjected to national drug regulations. If MPA was discovered to have major cytotoxic effects at small quantities, its availability and potential for abuse would constitute a serious health risk. In summary, the current data demonstrate that MPA does reduced cell viability at the highest tested concentration (1 mM). Menadione acts as a strong oxidative positive control (estimated IC_{50} = 0.02 mM). Future research should include the tested range beyond 1 mM to determine MPA's IC_{50} value.

CONCLUSION

In conclusion, the current data demonstrate that MPA significantly reduces BV2 microglial cell viability at the highest concentration tested (1 mM), although the IC_{50} was not reached within the tested concentration range of 0.0625 to 1 mM. Menadione acts as a strong oxidative positive control (estimated IC_{50} = 0.02 mM). Future research should include the tested range beyond 1 mM to establish the MPA's IC_{50} value.

LIMITATIONS

Several limitations should be acknowledged in the present study. First, the cytotoxicity of MPA was evaluated using an in vitro culture model, which simplifies the complex in vivo microenvironment and lacks cellular inter-communications. Second, the assessment of cell viability relied primarily on the MTT assay, which measures cellular metabolic activity and does not directly confirm cell death. Thus, further mechanistic studies such as flow cytometry for apoptosis and cell cycle analysis are warranted to fully delineate the mode of cell death. Lastly, while the BV2 provide a robust model, translating these findings to human physiology requires validation using human primary cells or in vivo animal models.

ACKNOWLEDGEMENT

This work was supported by the Polis Diraja Malaysia (PDRM) under Grant Number NN-2024-031.

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