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Research article

Synergistic effects of plasma-activated medium in combination with Baicalin against neuronal damage

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ABSTRACT

Neurodegenerative disorders are chronic conditions that progressively damage and destroy parts of the nervous system, and are currently considered permanent and incurable. Alternative strategies capable of effectively healing neuronal damage have been actively pursued. Here, we report the neuroprotective effects of baicalin (BA) combined with plasma-activated medium (PAM) against glutamate-induced excitotoxicity in SH-SY5Y cells. Through in vitro assays, the cell viability, inflammation, apoptosis, and oxidative stress were evaluated. The co-application of BA and PAM significantly enhanced cell viability, reduced pro-inflammatory markers (TNF-α and NF-κB), decreased apoptotic proteins (Bax and Caspase-3) and boosted antioxidative defenses (increased SOD activity and lowered ROS levels). This study confirms the potential of combining BA with PAM as an effective therapeutic strategy for mitigating the effects of excitotoxicity. PAM is a promising adjunct and potential drug delivery method in neuroprotective therapy, providing a new avenue for developing treatments for diseases characterized by neuronal damage.

1. Introduction

Neurodegenerative diseases are among the most widespread and disabling conditions globally [1]. The progressive loss of neurons characterizes these diverse disorders. Due to the limited regenerative capacity of nerve cells, nerve injuries, and neuronal loss often result in permanent functional impairments [2]. Chronic neurodegenerative conditions, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease [3]. Acute neuronal degeneration, such as spinal cord injury (SCI), hypoxia, and ischemia, can also lead to long-term neurodegenerative processes [4]. These conditions represent a significant unmet medical need in the world. Accumulating evidence suggests that mechanisms like glutamate excitotoxicity, oxidative stress, neuronal apoptosis, inflammatory responses, and mitochondrial dysfunction are associated with neurodegeneration [5–7]. Therefore, developing effective neuroprotective agents to prevent and control neurodegenerative diseases is critically important.

Clinically, drug therapy is a crucial approach to treating and preventing neurodegenerative diseases [8]. Commonly used medications address cognitive symptoms, anti-inflammatory drugs, and neurotrophic agents [9,10]. These drugs primarily aim to delay the progression of damage, inhibit the inflammatory microenvironment, and promote the differentiation and regeneration of nerve cells [11]. Baicalin (BA), a flavonoid compound derived from the dried root of *Scutellaria baicalensis,* is widely recognized for its diverse pharmacological activities within Traditional Chinese Medicine (TCM). There is substantial evidence indicating that baicalin possesses

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anti-inflammatory, anti-tumor, anti-apoptotic, and antioxidant properties [12,13]. Due to its ability to penetrate the blood-brain barrier (BBB) and enter the cerebrospinal fluid, BA exhibits potential for neuroprotection and cognitive enhancement across various models of neuronal damage [14–16]. However, using a single drug may be insufficient to fully control disease progression or alleviate symptoms, particularly in complex conditions such as cancer or neurodegenerative diseases [17,18]. Researchers have long been dedicated to developing new drugs and other auxiliary methods to address the limitations of traditional medications [19].

Plasma, the fourth state of matter after solid, liquid, and gas, consists of active species, charged particles, ultraviolet (UV) radiation, and electric fields. These components enable plasma to influence the biological functions of tissues and cells directly or indirectly. This has led to the rapidly expanding use of cold atmospheric plasma (CAP) in fields including biology, medicine, and agriculture [20]. In recent years, an increasing number of studies have explored the application of CAP in the pharmaceutical field [21]. Compared to traditional drug synthesis methods that require harsh conditions, long reaction times, and complex extraction processes, CAP can more rapidly and easily enhance the biological activities of natural ingredients or produce beneficial byproducts. CAP can be used to treat solutions and produce plasma-activated liquids. One approach involves effectively infusing these into culture media to create what is known as "plasma-activated medium" (PAM). Some research suggests that combining CAP with drugs can synergistically enhance the efficacy [22,23]. Consequently, plasma-activated medium has broad application in cancer and dental treatments [24]. In these contexts, they synergize with medications to achieve high selectivity, enhance intracellular accumulation of drugs as well as reduce inflammatory responses [25–27]. Under the modulation by PAM, some compounds may undergo functional enhancements, thereby achieving diverse effects such as optimizing drug delivery systems, extending drug efficacy, and boosting antioxidant activities [28–31]. In particular, research demonstrates that CAP treatment can enhance the therapeutic properties of flavonoid compounds by modifying polyphenols to generate primary and secondary products [32]. These modified ingredients exhibit various biological effects, including anti-diabetic properties, highlighting CAP's versatile and impactful nature in pharmaceutical development [33,34]. As previously mentioned, BA also holds potential in neural protection. However, to the best of our knowledge, no relevant studies have yet

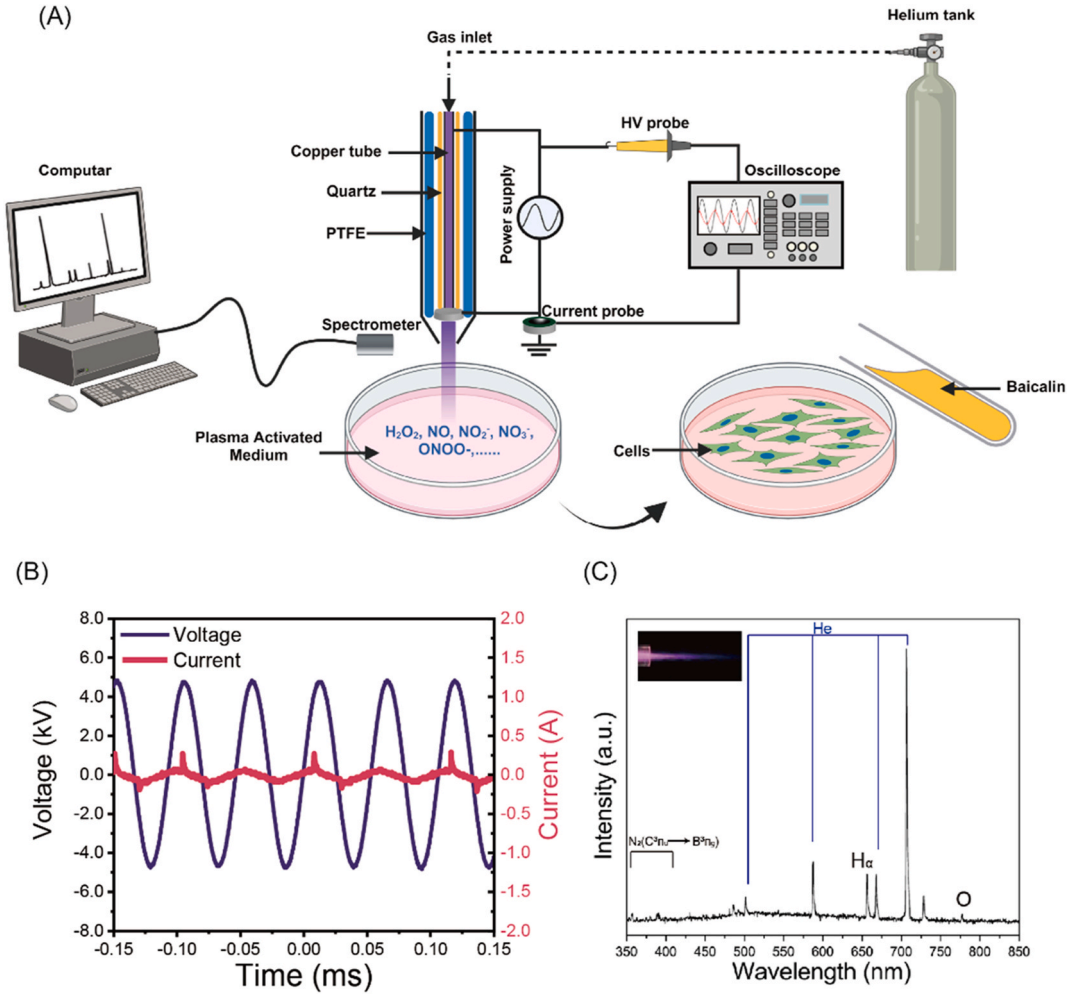

Fig. 1. Experimental setup and analysis of the plasma jet source. (A) Schematic diagram of the experimental setup. **(B)** Voltage and current waveform. **(C)** Optical emission spectroscopy spectrum of plasma jet at wavelengths ranging from 350 to 850 nm.

investigated the synergistic effects between PAM and BA. A significant gap remains in understanding the specific mechanisms by which PAM interacts with drugs, particularly in the context of neural damage. Investigating the synergistic effects of PAM combined with BA offers a promising approach to enhance neuroprotective treatments and deepen our understanding of the underlying synergistic mechanisms of CAP in combating neuronal damage.

In the study, we aim to investigate the synergistic neuroprotective effects of BA and PAM on neuronal cells under conditions of glutamate-induced excitotoxicity, a prevalent pathological feature of many neurodegenerative diseases. A series of in vitro assays were conduct to monitor changes in cell viability, inflammatory markers, and apoptotic indicators within SH-SY5Y neuroblastoma cells. Employing quantitative analysis and molecular biology techniques, the mechanistic pathways through which BA and PAM confer neuroprotection were further investigated. This study not only seeks to deepen the understanding of CAP's role in enhancing the efficacy of neuroprotective agents but also to establish new therapeutic strategies for treating neurodegenerative diseases.

2. Results

2.1. Plasma-activated medium generation and plasma characterization

Fig. 1A depicts a schematic of the plasma jet source. The setup uses Polytetrafluoroethylene (PTFE) as an insulating shield, with a tube covered by copper and linked to the power supply. The working gas is fed from the tube tip. PAM is produced by exposing plasma to DMEM without FBS and antibiotics. Specifically, 3 mL of DMEM was activated for 2 min at a flow rate of 2.0 standard liters per minute while exposed to helium plasma. Following this, antibiotics and FBS were added, and the PAM was immediately introduced to the cells. Baicalin was then added to the fully-supplemented medium. As illustrated in Fig. 1A, when the device is discharging, a high-voltage probe measures the voltage at both ends of the plasma generator, and a current sensor connected in series in the discharge circuit of the reactor measures the discharge current.

The recorded jet discharge voltage and current waveforms are shown in Fig. 1B. The voltage signal exhibits a stable sine waveform. During each positive and negative half-cycle of plasma discharge, numerous narrow discharge pulses appear in the current waveform. However, notable distinctions exist in the discharge patterns between the two half-cycles. The amplitude of the current during the positive half-cycle slightly surpasses that of the negative half-cycle. This discrepancy arises from the accumulation of charges transmitted through the micro-discharge channel on the surface area of the dielectric plate, forming a wall charge layer. This layer generates an internal electric field under the influence of wall charges, resulting in asymmetric positive and negative half-cycle current waveforms. Electrical measurements indicate that the operation of the plasma discharge device is relatively stable.

Optical emission spectroscopy (OES) is a widely utilized method for studying active substances in plasma, prized for its simplicity, ease of operation, and non-invasiveness. As shown in Fig. 1C, we investigate the emission lines of various atomic species, mainly focusing on neutral helium (He) and oxygen (O) atoms. The emission lines of excited He atoms are observed at wavelengths of 587.6 nm and 667.8 nm, corresponding to transitions from He ($3^3D \rightarrow 2^3P$) and He ((3 [1]$D \rightarrow 2^1P$), respectively [35]. These transitions predominantly occur due to collision ionization between ground-state helium atoms and inelastic electrons. Additionally, a spectral line at 501 nm arises from the transition of He ($3p^1$P-$2s^1$S) [36].

For oxygen, the spectral line at 777 nm emerges from the transition of O ($3p^5P \rightarrow 3s^5S$), which can be generated via two main processes: direct collision ionization of high-energy electrons with ground state O_2 molecules and Penning reaction involving metastable helium atoms (He*) and O_2. Specifically, He* species, including He(2 [1]S) and He(2 [3]S), play a crucial role in facilitating the Penning reaction [37].

$$He^* + O_2 \rightarrow He + O(3p^5P) + O$$

In the spectral range spanning 350–500 nm, we also detect faint emission lines attributed to nitrogen species, namely N_2 and N_2^+ [38]. The intensities of these lines are relatively weak, given that helium serves as the primary working gas in the discharge area. The

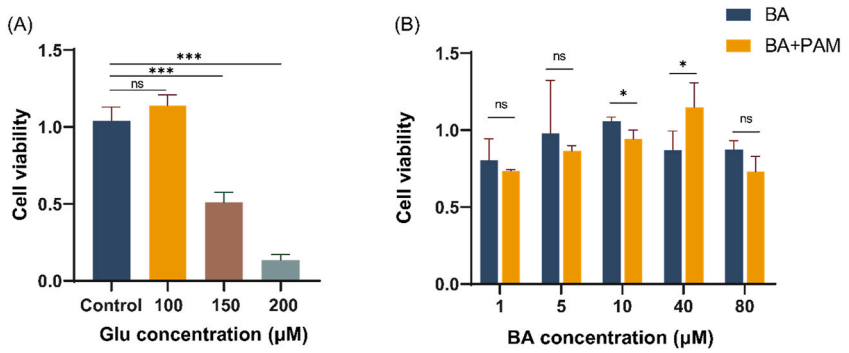

Fig. 2. Dose-response effects of Glu and BA, with and without PAM, on SH-SY5Y cell viability. (A) Cell viability after inducing with different concentrations of Glu. **(B)** Cell viability of SH-SY5Y cells with Glu excitotoxicity damage after treatment with BA and BA + PAM. The experiment was repeated three times. Data are expressed as the mean ± SD. *P < 0.05, ***P < 0.001.

ionization and excitation of nitrogen molecules from the ambient air are minimal, resulting in the subdued spectral contribution of nitrogen within this wavelength region.

This comprehensive analysis provides valuable insights into the mechanisms underlying the emission spectra of helium, oxygen, and nitrogen species in plasma environments, aiding in the understanding and characterizing plasma processes.

2.2. Establishing concentration parameters for neuroprotection study

Glutamate (Glu) is a non-essential amino acid and the primary excitatory neurotransmitter in the central nervous system. Excessive excitatory activation of glutamate can lead to neurotoxicity, which is implicated in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [39,40]. Glu is frequently employed to simulate excitatory toxicity in SH-SY5Y cells by inducing oxidative stress, a mechanism relevant to both acute and chronic neural injuries. This approach is widely utilized in related research studies [41–43].

Cell viability assays were performed to determine the appropriate concentrations of Glu for inducing excitotoxicity. Three concentrations (100, 150, and 200 μM) were selected for testing. As depicted in Fig. 2A, cell viability at 24 h post-treatment was 1.14, 0.51, and 0.14 relative to the control group, respectively. Notably, a Glu concentration of 150 μM halved the cell viability, effectively reducing it to 50 % of the control. This significant reduction in viability is ideal for establishing a stable and consistent neurotoxicity model. Therefore, 150 μM was chosen as the optimal concentration to establish the excitotoxicity model in SH-SY5Y cells for subsequent studies. This concentration reliably induces a moderate yet substantial level of toxicity, which is crucial for exploring protective interventions in future experiments.

To explore the therapeutic potential of BA in combination with PAM following excitotoxic injury, we initially treated SH-SY5Y cells with various concentrations (1, 5, 10, 40, 80 μM) of BA after inducing excitotoxicity with Glu for 24 h in Fig. 2B. This preliminary step was crucial to determine the optimal concentration of BA that maximizes cell viability, providing a necessary baseline for subsequent comparisons.

When BA is combined with PAM, a synergistic effect may occur, altering the required concentration of BA to achieve optimal cell protection. This synergistic interaction could reduce or shift the effective concentration needed, achieving the same or enhanced cell viability. As shown in Fig. 2B, a dose-dependent increase in cell viability was observed with increasing BA concentrations when combined with PAM. The viability peaked at 40 μM, suggesting that this concentration provides the most effective protection against the excitotoxic effects induced by Glu. Consequently, 40 μM was identified as the optimal BA concentration for further studies to investigate the synergistic effects of BA and PAM in this model. At higher concentrations (80 μM), adding PAM does not significantly

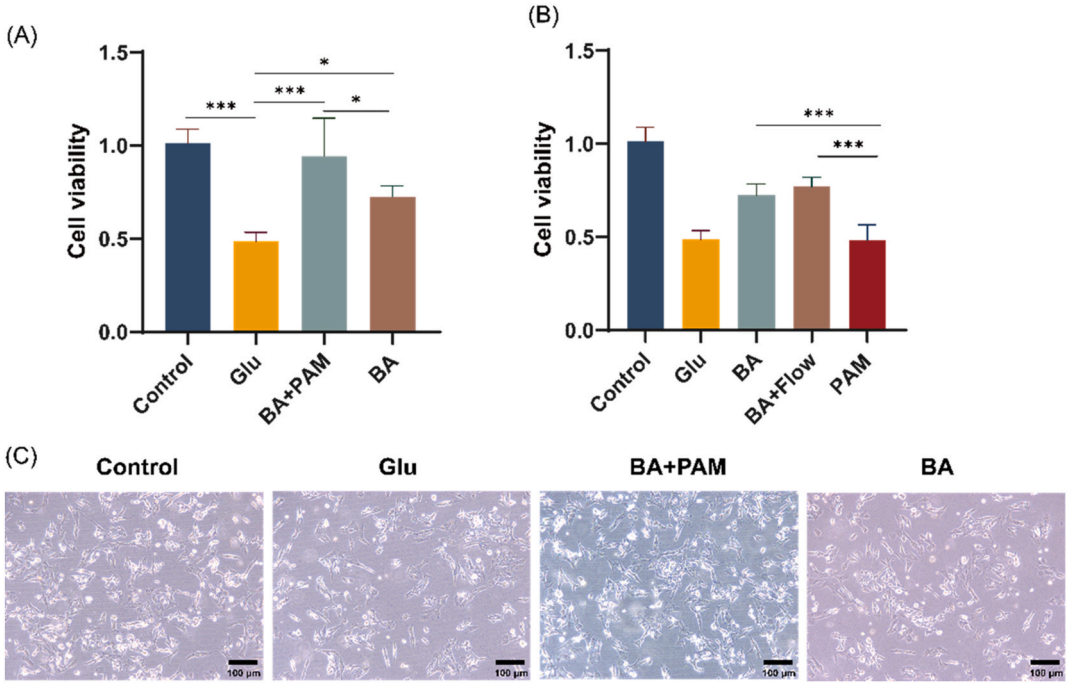

Fig. 3. Evaluating the neuroprotective effects of BA and PAM on SH-SY5Y Cells post-Glu treatment. (A) Effect of BA and BA + PAM on cell viability in SH-SY5Y cells. **(B)** Comparison of cell viability responses to BA, BA + Flow (exposed only to helium gas flow), and PAM (activated by helium plasma) in Glu induced SH-SY5Y Cells. **(C)** The morphological analysis of SH-SY5Y cells under different treatment conditions was captured at 20× magnification. Scale bar = 100 μm. The experiment was repeated five times. Data are expressed as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

improve the protective effect of BA. This could suggest that the protective effect of BA + PAM plateaus or even decreases at higher doses, which is an essential consideration for therapeutic applications.

2.3. BA and PAM combined strategy increased the cell viability of SH-SY5Y

To further investigate the protective effects of BA and its interaction with PAM, we extended our analysis to the cell viability outcomes post-treatment. In Fig. 3A, treatment with BA leads to a significant enhancement in cell viability relative to the Glu group, confirming BA's protective properties against glutamate-induced cytotoxicity, as supported by multiple studies. Notably, the combination of BA and PAM results in even higher cell viability, increasing from 0.73 with BA alone to 0.94 with BA + PAM ($P < 0.05$). This improvement indicates a synergistic interaction between BA and the active components within PAM. The SH-SY5Y control cells in normal conditions exhibit typical epithelioid cell morphology (Fig. 3C). After treatment with Glu, most cells shrink and become round in shape, indicative of cell damage. In contrast, cells treated with BA show an alleviation of Glu-induced excitotoxicity, maintaining a morphology similar to that of the control cells. Cells treated with BA + PAM exhibit an intermediate morphology between the Glu-induced and BA-treated cells, suggesting partial protection against Glu-induced damage.

Although BA + PAM showed promising results, some studies have indicated that PAM alone can treat certain diseases. Therefore, to exclude related effects, we used PAM alone to treat toxicity caused by Glu. Interestingly, treatment with PAM alone did not result in a significant change in cell viability compared to the Glu group (Fig. 3B), indicating that PAM neither exhibits intrinsic cytotoxicity nor confers a protective effect on the cells under the experimental conditions employed. Additionally, cells are treated with BA and helium gas flow without plasma activation, the viability mirrors that of BA treatment. This implies that the helium flow alone does not contribute significantly to cell viability, ruling out the influence of gas flow on BA and PAM's efficacy.

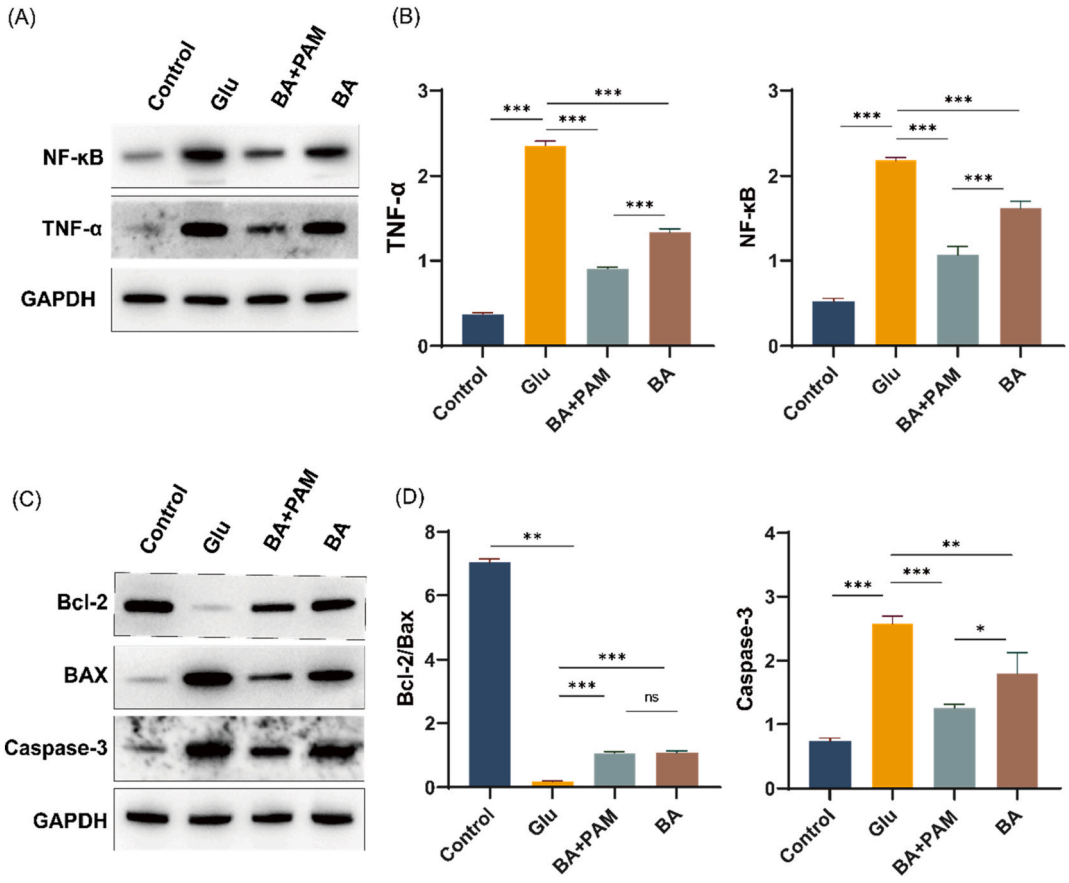

Fig. 4. Effect of BA + PAM combined strategy on inflammatory and apoptosis proteins. (A) Western blot assay of the inflammatory response (NF-κB and TNF-α) proteins. **(B)** Quantitative analysis of NF-κB and TNF-α protein expression. **(C)** Western blot assay of the apoptosis response (Bcl-2, BAX and Caspase-3) proteins. **(D)** Quantitative analysis of Bcl-2/BAX ratio and Caspase-3 protein expression. The experiment was repeated three times. Data are expressed as the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

2.4. BA and PAM combined strategy enhanced anti-inflammatory and anti-apoptotic responses in SH-SY5Y cells

Western blot analysis was performed on cells post-treatment further to evaluate the therapeutic effects of the CAP + PAM combined strategy. NF-κB is a critical transcriptional regulator of the inflammatory response and is essential in regulating inflammatory signaling pathways [44]. TNF-α, a cytokine, acts as a crucial mediator in these pathways by activating NF-κB, among other transcription factors, thereby influencing the expression of various inflammatory genes. Thus, the inflammatory response of SH-SY5Y cells was assessed by detecting the expression levels of proinflammatory factors in Fig. 4A and S1. The expression of TNF-α and NF-κB is significantly higher in the Glu-induced group compared to the control, suggesting an enhanced inflammatory response and associated cellular damage [45] (Fig. 4B). Researchers have confirmed that treatment with BA dramatically inhibits the NF-κB signaling pathway [46]. Consistent with these findings, our study shows that BA alone reduces levels of both NF-κB and TNF-α. Furthermore, the combination of BA and PAM results in a more substantial decrease in the expression levels of these proteins, suggesting an enhanced mitigating effect on inflammation. This indicates that while BA is effective independently, it is more potent when used with PAM.

Subsequently, the Bcl-2/BAX ratio and Caspase-3 expression, critical indicators of cell survival, were assessed. As shown in Fig. 4C and S2, the BA and BA + PAM treatment drastically increases the Bcl-2/BAX ratio that Glu had diminished. Caspase-3 levels, which were significantly upregulated in the Glu-induced group, showed a substantial decrease following BA intervention (Fig. 4D). Additionally, the BA combined with the PAM intervention group further reduced these levels, underscoring the synergistic protective effect of the combined BA and PAM treatment.

In summary, the treatments with BA + PAM are more effective in reducing markers of inflammation and apoptosis induced by Glu. These results confirm the protective effects of BA, especially when combined with PAM, against Glu-induced cellular stress and apoptosis in SH-SY5Y cells.

2.5. BA and PAM combined strategy boosted antioxidative defense in SH-SY5Y cells

Many studies have demonstrated that baicalin can modulate inflammatory and apoptotic responses triggered by oxidative stress [47,48]. ROS are both contributors to and indicators of oxidative stress. The balance between ROS production and antioxidant defenses is crucial for maintaining cellular function and preventing oxidative damage. Thus, intracellular ROS levels were measured using flow cytometry. Fig. 5A and B illustrated ROS levels in SH-SY5Y cells under various conditions. The control group shows baseline ROS

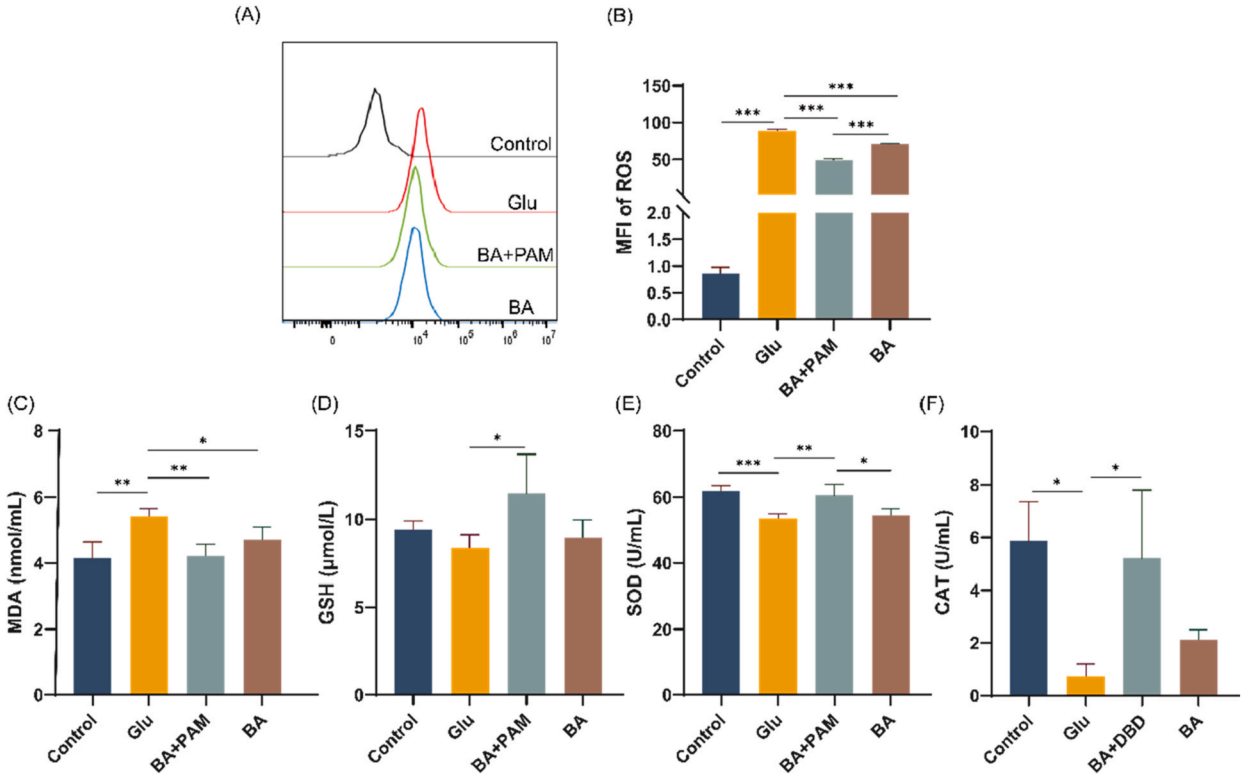

Fig. 5. Intracellular ROS content and oxidative stress indicators level. (A) Flow cytometry analysis of ROS levels using DCFH-DA assay in SH-SY5Y cells under different treatments. **(B)** Quantitative comparison of mean fluorescence intensity of ROS. **(C)** MDA **(D)** GSH **(E)** SOD **(F)** CAT activities in response to treatments, were measured by corresponding assay kits. The experiment was repeated three times. Data are expressed as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

levels, with a marked increase in the Glu-induced cells, indicating enhanced oxidative stress. In contrast, BA treatment alone and, more effectively, BA + PAM treatment significantly lower ROS levels, demonstrating the antioxidative effects of the combined treatment.

Antioxidant capacity is vital in neurodegenerative diseases because it helps to mitigate oxidative stress, thereby protecting neurons from damage and possibly slowing disease progression. Studies have demonstrated that Glu-induced toxicity was significantly prevented by treatment with antioxidants [49,50]. Building on the demonstrated ability of BA to modulate oxidative stress responses, subsequent analyses focused on a detailed examination of critical antioxidants within the cellular environment.

This study quantitatively analyzed levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) as critical catalysts in scavenging reactive oxygen species (ROS), and malondialdehyde (MDA) as a marker of peroxidation-induced damage. MDA levels, which were elevated in Glu-induced cells, were notably reduced by BA and BA + PAM, suggesting adequate protection against oxidative damage (Fig. 5C). However, the differences between BA and BA + PAM were not statistically significant. Similarly, while there were no significant changes in GSH levels among the Control, Glu, and BA groups, BA + PAM was significantly higher than Glu (Fig. 5D). Meanwhile, SOD activity was enhanced in treatments with BA (54.49 U/mL) and BA + PAM (60.56 U/mL) compared to Glu alone, indicating that BA + PAM has a higher SOD activity than BA alone (Fig. 5E). However, CAT activities did not show significant differences between the BA and BA + PAM groups, indicating that the antioxidant capacity enhancement in the BA + PAM group may primarily involve mechanisms other than catalase activity (Fig. 5F). This comprehensive antioxidative response highlights the potential of BA + PAM in combating oxidative stress more effectively than BA alone. In short, the combination of PAM and BA alters the environment in damaged cells by triggering self-antioxidant enzymes and enhancing the antioxidant capacity of BA [46,51].

3. Discussion

In recent years, the neuroprotective effects of flavonoids have gained attention in various neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and cerebral ischemia [52]. These effects are attributed to their anti-apoptotic, anti-inflammatory, anti-oxidative stress, anti-excitotoxicity, neurogenesis-promoting, and cell differentiation-promoting properties. Baicalin (BA), a prominent flavonoid derived from *Scutellariae Radix*, has been extensively studied and has shown promise in reversing changes in apoptosis-related proteins, inflammatory cytokines, and NF-κB pathway proteins in the spinal cord. These proteins include Bax, Bcl-2, Caspase-3, TNF-α, IL-1β, IL-6, and NF-κB [53,54]. However, challenges such as the blood-brain barrier (BBB) and age-related modifications in neuronal membranes can reduce the efficacy of drugs like BA [55]. Furthermore, increasing the dosage to counteract these effects may also heighten the risk of secondary, undesired effects [56]. Therefore, developing strategies that enhance the efficacy and delivery of neuroprotective drugs to target these diseases effectively is crucial. Our findings build upon the findings of previous research, illustrating that BA can significantly mitigate excitotoxic effects, a common pathological feature in disorders like Alzheimer's and Parkinson's disease. Notably, our results underscore BA's potential when combined with PAM, extending its applicability beyond traditional anti-inflammatory and antioxidant roles.

This study leverages PAM's unique properties to enhance BA's therapeutic efficacy, particularly against glutamate-induced excitotoxicity—a critical mediator of neuronal damage in both acute and chronic neurodegeneration. Our findings reveal that the most effective synergistic interaction between BA and PAM occurs at a concentration of 40 μM. This differs from the optimal concentration of BA alone, which is 10 μM, indicating that the synergy modifies the BA concentration needed to achieve optimal cell protection. This synergy appears to plateau at higher concentrations, suggesting a finely tuned balance between efficacy and potential saturation of therapeutic effects, a novel finding of significant clinical relevance. This highlights a targeted approach that could optimize treatment paradigms in combination therapies.

The synergistic effects observed in our study between BA and PAM offer compelling evidence for the modulation of both inflammatory and apoptotic pathways in neurodegenerative disease models. Inflammatory markers such as TNF-α and NF-κB significantly increase under Glu-induced stress, contributing to neuronal damage. BA reverses the upregulation of these markers. Adding PAM further enhances BA's effects, leading to a more pronounced decrease in TNF-α and NF-κB levels. This suggests that PAM may improve BA's bioavailability or efficacy by interacting with cellular pathways to augment its anti-inflammatory capabilities.

Concurrently, our study demonstrates the anti-apoptotic effects of BA and PAM, particularly evidenced by changes in vital apoptotic markers. While treatments with BA alone and BA + PAM increased the Bcl-2/BAX ratio, the more notable effect was the reduction of Caspase-3 levels. Caspase-3, significantly elevated in the glutamate-induced group, was substantially reduced following treatment with BA, and this reduction was further enhanced in the BA + PAM group. This underscores the synergistic protective effect of the combined treatment, emphasizing its significant potential in inhibiting the apoptotic cascade and promoting neuronal survival. These effects mitigate cell death and modify the cellular environment to foster cell survival and resilience against oxidative stress—a common mediator of cellular damage in neurodegenerative diseases.

Moreover, the enhanced antioxidative response facilitated by the BA and PAM combination underscores the crucial role of oxidative stress mitigation in neuroprotection. The significant reduction in ROS levels, the associated decrease in oxidative damage markers such as MDA, and the increase in antioxidant enzyme activities suggest that this strategy effectively counters the biochemical pathways leading to cell death in neurodegeneration. This points to the therapeutic relevance of targeting multiple pathways involved in cell death and survival, which could be particularly advantageous in multifactorial diseases like Alzheimer's and Parkinson's.

The underlying mechanism behind the observed synergistic effects between PAM and BA likely involves their interaction at the cellular level. Such effects can be attributed to the increased intracellular accumulation of drugs, a phenomenon supported by existing studies that highlight how PAM enhances the efficacy of pharmaceutical agents through this mechanism. Exploratory research has demonstrated that plasma-produced reactive radicals can transiently permeabilize cell lipid bilayer membranes, facilitating the passage of molecules into cells [57,58]. Notably, PAM causes less damage to healthy cells and has been demonstrated to modify cell

membrane structures and chemical properties by generating reactive oxygen and nitrogen species (RONS) [59,60]. By altering cell membrane structures, PAM facilitates a more significant accumulation of drugs within cells, thereby amplifying their therapeutic effects [61–63]. Specifically, these alterations may increase the stability and delivery of BA to damaged cells. Such improvements could significantly enhance BA's bioavailability and efficacy. Our findings also suggest that the combination may improve the expression of genes encoding antioxidant enzymes such as superoxide dismutase and boost glutathione levels, providing a dual mechanism that mitigates oxidative stress and promotes cellular health [51,64,65].

4. Conclusion

In conclusion, this study substantiates the neuroprotective potential of BA when synergistically combined with PAM, providing significant evidence that supports its use against excitotoxicity commonly associated with neurodegenerative disorders. The notable enhancements in cellular resilience against oxidative stress, inflammation, and apoptosis demonstrate the therapeutic value of this combination. These results suggest that utilizing the combined properties of PAM and BA offers a viable and promising approach for improving drug delivery and treating conditions associated with neurodegenerative changes. Future research should focus on clinical translations and deeper mechanistic understanding to optimize and broaden the therapeutic applications of this combination.

5. Methods

5.1. PAM generation

3 mL of DMEM without FBS and antibiotics was activated by CAP for 2 min at a flow rate of 2.0 standard liters per minute while being exposed to pure helium gas. Pen Strep and FBS were added, and the PAM was immediately added to the cells. Baicalin (purity ≥98 %), obtained from Aladdin, was added to a fully-supplemented medium.

5.2. Cell culture and drug intervention

SH-SY5Y cells were obtained from iCell and cultivated using DMEM medium (Gibco, Grand Island, NY, USA) containing fetal bovine serum (10 %), penicillin (100 units/mL) and streptomycin (100 μg/mL) in a 5 % CO_2 humidified incubator at 37 °C. The suspension containing SH-SY5Y (cell concentration 1×10^6/mL) was added to a 96-well culture plate at 100 μL per well. A stable neurotoxicity model was established by treating the cells with 150 μM glutamate for 24 h, which reduced cell viability to 50 % of the control. Culturing continued for 24 h. The culture medium was removed before the intervention.

To simulate cellular responses following neural injury, we introduced glutamate (Glu) to the culture medium at a concentration of 150 μM for 24 h. This approach effectively reduced cell viability to 50 % compared to the control group, thereby establishing a stable model of neurotoxicity.

5.3. Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8). After 24 h of treatment with the test compounds, SH-SY5Y cells were washed with PBS. Subsequently, 100 μL of DMEM medium was added to each well. This was followed by adding 10 μL of 10 % CCK-8 solution to each well, and the plate was incubated at 37 °C for 1.5 h in a water bath. Absorbance at 450 nm was measured using a microplate reader.

5.4. Western blot assay

The main antibodies were TNF-α, NF-κB p65, Bcl-2, Bax, caspase-3 (1:1000, Energenesis Biomedical, China) and GAPDH(Proteintech, USA).Cells were lysed using RIPA buffer (Beyotime, China), centrifuged (12,000 rpm, 4 °C, 10 min), and the supernatant containing cellular proteins was harvested. Protein concentrations were determined using the BCA assay, with concentrations ranging from 3 to 6 μg/μL. A total of 30 μg of protein per sample was loaded onto the gel. Protein concentrations were normalized, mixed with loading buffer, and denatured (100 °C, 10 min). Samples were subjected to SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked with skim milk (room temperature, 2h), incubated with primary antibodies overnight at 4 °C. Following primary antibody incubation, membranes were washed with PBST, incubated with secondary antibody (1:5000 dilution) for 1h, and washed again. Protein bands were visualized using an ECL kit and quantified with ImageJ software.

5.5. Flow cytometry

After 24 h of intervention with different preparations in the cell model, a medium containing 2′,7′-dichlorofluorescin diacetate (DCFDA) was added to the cells. Typically, the final concentration of DCFDA is 10 μM, with an incubation period of 20–30 min at 37 °C. Following incubation, cells were washed once or twice with PBS to remove any non-internalized DCFDA. Cells were then trypsinized, collected, and resuspended in PBS. The cell suspension was transferred to flow cytometry tubes, and DCF fluorescence intensity was measured using a flow cytometer (Beckman Coulter, CytoFLEX LX). The software (FlowJo) associated with the flow cytometer was used to analyze the fluorescence intensity of DCF within the cells to assess the level of reactive oxygen species (ROS) production.

5.6. Oxidative stress level

After washing the cells twice with phosphate-buffered saline (PBS), they were carefully scraped off using a cell scraper. The cell suspension was then centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and the cell pellet was retained. Subsequent biochemical assays were conducted using a commercial kit provided by the Nanjing Jiancheng Bioengineering Institute, China. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) in the tissue homogenate were measured according to the manufacturer's instructions.

5.7. Statistical analysis

All the experimental results were analyzed by GraphPad Prism 9.0 and Origin 2023. Data were expressed as mean ± standard deviation (SD). Statistical differences were analyzed with a one-way analysis of variance followed by the least significant difference post hoc test. $p < 0.05$ was taken as statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001).

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Data availability statement

Data are available on request/reasonable request. The author, Jiwen Zhu, will provide the data generated from this study upon direct email request to zhujiwen@mail.ustc.edu.cn.

CRediT authorship contribution statement

Jiwen Zhu: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Data curation. **Qi Liu:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Yan Chen:** Investigation, Formal analysis. **JiaMing Zhang:** Visualization, Resources. **Qinghua Xu:** Writing – review & editing, Methodology. **Zhengwei Wu:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36079.

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