Research Square

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Mitochondrial Dysfunction and Antiproliferative Effects of α-Mangostin Extracted from the Pericarp of the Mangosteen Fruit (Garcinia mangostana L.) on Rat C6 Glioma Cells

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Abstract

Objective: This study aims to evaluate the effects of α-mangostin, a xanthone present in the pericarp of mangosteen (Garcinia mangostana L.), on C6 glioma cells, an in vitro model for glioblastoma.

Methods: The study was conducted using an in vitro model with C6 glioma cells. The antioxidant activity of α-mangostin was measured using the IC_{50} value for DPPH free radical scavenging activity. Cytotoxicity was assessed using the MTS assay. DNA fragmentation analysis was performed to determine DNA damage in C6 cells. Additionally, changes in mitochondrial morphology and membrane potential in C6 cells upon exposure to α-mangostin were evaluated using mitochondrial fluorescence staining and membrane potential measurement.

Results: The results showed that the antioxidant activity of α-mangostin increased in a concentration-dependent manner, with an IC_{50} value for DPPH free radical scavenging activity of 67.55 ± 0.91 μg/mL. The proliferation of C6 cells decreased as the concentration of α-mangostin increased, demonstrating cytotoxicity with an IC_{50} value of 6.57 ± 0.199 μg/mL. α-mangostin also induced DNA damage in C6 cells, as evidenced by DNA fragmentation analysis. Furthermore, α-mangostin from mangosteen pericarp altered mitochondrial function and morphology in C6 cells in a concentration-dependent manner.

Conclusion:α-mangostin extracted from mangosteen pericarp exhibited significant effects on C6 glioma cells. This study underscores the promising preclinical potential of α-mangostin as a multitarget therapeutic agent in the treatment of glioma.

Background

Gliomas are the most common type of primary brain tumors, accounting for over 45% of central nervous system tumors and posing a significant threat to human health [1]. The median survival time for patients with newly diagnosed glioblastoma is usually less than 15 months, and the 5-year survival rate for these patients is approximately 5% [2]. Chemotherapeutic agents mentioned for the treatment of glioma, such as Temozolomide, Enzastaurin (LY317615), and Erlotinib (Tarceva), along with immunotherapies and gene therapies, are being researched to improve treatment efficacy. Notably, Temozolomide (TMZ) is the leading chemotherapeutic agent for glioma treatment; however, its efficacy is often limited by the durability of chemoresistance responses [3]. Therefore, the development of improved drugs is essential to prevent the recurrence and distant metastasis of GBM and to prolong survival in glioma patients

Mangosteen (Garcinia mangostana), often referred to as the "queen of fruits" due to its delightful taste, is cultivated in Southeast Asian countries, including Indonesia, Myanmar, Cambodia, Thailand, and Vietnam [4]. Besides being a delicious fruit, the fruit and rind of the mangosteen have long been used for medicinal purposes, regarded as a folk medicine with numerous pharmacological properties. Extracts from the mangosteen rind contain a family of tricyclic isoprenylated polyphenols known as xanthones, which are the most abundant components in mangosteen extracts [5]. It has been reported that xanthones in the mangosteen rind exhibit various bioactivities and pharmacological properties, including

anti-tumor capabilities, making xanthones potential chemotherapeutic agents of significant interest to scientists. These agents have been shown to regulate cell division and growth, inflammation, and metastasis in different stages of carcinogenesis. Moreover, most studies evaluating xanthones from mangosteen focus on the compound α-mangosteen, with particular emphasis on its anti-cancer and chemotherapeutic properties. Previous reports have demonstrated that α-mangostin exhibits antioxidant [6], antibacterial [7] anti-inflammatory [8], and anti-cancer effects [9] such as against breast cancer [10], colon cancer [11], prostate cancer, and skin cancer.

Common mechanisms in cancer treatment include inhibition of cell proliferation, induction of DNA damage, and disruption of mitochondrial function leading to apoptosis. A cytotoxic agent can act in various ways, such as targeting specific molecules (e.g., receptors, enzymes) or disrupting cellular structures, causing cellular stress and cytotoxicity. Recently in 2021, scientists conducted toxicity tests of α-mangostin on various cancer cell lines, achieving promising results that lay the foundation for further research into its toxicity on neural cancer cell lines [10]. Genotoxicity and mitochondrial membrane potential disruption in cancer cells are other critical targets for evaluating cancer cell cytotoxicity. Genetic toxicity refers to the ability of a substance to cause breaks or alterations in genetic material (DNA or chromosomes). Accurate assessment of genetic damage is valuable for understanding the cellular condition, as DNA damage is often implicated in the initiation and progression of various cancers. Mitochondria plays a crucial role in maintaining cellular homeostasis, including energy synthesis, redox regulation, and cell proliferation and apoptosis regulation in cancer cells. In cancer cells, the shift from oxidative phosphorylation to glycolysis impairs mitochondrial function, increasing ROS levels, which are significantly associated with oncogenic signaling. The mitochondrial membrane potential is an indicator used to assess mitochondrial functions. The electrochemical gradient caused by the imbalance of H + between the intermembrane space and the matrix affects the mitochondrial membrane potential [12]. Recent reports have investigated the impact of plant extract constituents like epigallocatechin gallate (EGCG) from green tea, resveratrol from grape skins, and gingerol from ginger on cancer cell mitochondria. These compounds affect mitochondrial-related proteins involved in cell death activation or mitochondrial membrane potential destabilization [13, 14]. To date, there have been no studies on the effects of mangosteen pericarp extract on DNA and mitochondrial damage in C6 glioma cells (an in vitro model for glioblastoma multiforme – a highly malignant tumor with a high mortality rate, widely used to analyze glioma characteristics like growth, invasion, migration, and angiogenesis [15]. Therefore, this study aims to evaluate the inhibitory effects of mangosteen pericarp extract on cell proliferation, cytotoxicity, DNA damage, and mitochondrial function in mouse glioma C6 cells.

Results

Antioxidant capacities of α-mangostin

The antioxidant activity of α-mangostin was evaluated using its DPPH radical scavenging activity. When interacting with DPPH, antioxidants transfer an electron or a hydrogen atom to DPPH, thereby

neutralizing its radical properties. Consequently, the absorbance at a wavelength of 517 nm is proportional to the residual DPPH amount. As shown in Fig. 1, the free radical scavenging activity of α-mangostin increases as its concentration escalates from 2 µg/mL to 100 µg/mL. The IC_{50} values of α-mangostin and ascorbic acid are 67.55 ± 0,91 µg/mL and 28.4 ± 0,72 µg/mL. The findings of the study demonstrate that α-mangostin is an efficacious compound capable of functioning as an antioxidant by supplying hydroxyl radicals.

Effect of extract on the viability of C6 cells

The cytotoxicity of α-mangostin on C6 glioma cells was evaluated by using the MTS assay, and cell morphology was observed under a stereomicroscope, yielding images such as those shown in Fig. 2.

Cells treated with a concentration of 0.5 µg/mL did not show any inhibitory effect on proliferation compared to the control sample. However, at the concentration of 5 µg/mL of the extract, cells began to exhibit inhibited proliferation after 24 hours. The cytotoxicity of α-mangostin correlates with the treatment concentration, with higher concentrations resulting in more cell death. After 24 hours at a concentration of 10 µg/mL of α-mangostin, cells started to round up, although some cells with adhesive capabilities remained. At a concentration of 15 µg/mL, all cells became rounded, and at 25 µg/mL, a significant number of dead cells were observed.

C6 cells were treated with α-mangostin at various concentrations for 72 hours, and the cytotoxicity assay was conducted using the MTS method. The results perform in Fig. 3. demonstrated a dose-dependent decrease in C6 cell viability. The cell viability decreased slightly to 92.33 ± 3.249% at 0.5 µg/mL concentration and drastically dropped from 5 µg/mL concentration to 43.19 ± 3.079%, further declining to 6.32 ± 0.672% at 10 µg/mL concentration. At 25 µg/mL concentration, cell viability reduced to only 1,23 ± 0.417%. The results from the graph allowed for the calculation of the half-maximal inhibitory concentration (IC_{50}) as 4.67 ± 0.199 µg/mL with a significance value of $*P < 0.05$.

Effect of α-mangostin on cellular DNA fragmentation

Comet assay was employed to evaluate the extent of DNA damage in C6 cell nuclei following treatment with different concentrations of α-mangostin for 24 hours. Fluorescence staining revealed that α-mangostin affected cell nuclei. Negative controls showed round, evenly stained nuclei with no impact and positive controls treated with 500 µM H_2O_2 exhibited DNA tail formation. DNA damage in cell nuclei became apparent at a concentration of 5 µg/mL (Fig. 4). The average percentage of DNA tails was calculated and presented in the graph for the extract-treated samples, showing a 17.95 ± 6.277% increase compared to the negative control (2.17 ± 1.543%) with a significance level of $P < 0.05$ (Fig. 5)

Effect of α-mangostin on cell mitochondria

Function and quality of mitochondria are crucial determinants of cell fate. Morphological changes in mitochondria may represent early adaptive responses of cells when exposed to a compound. In this study, we utilized MitoTracker™ Orange CMTMRos – a fluorescent dye to assess mitochondrial

morphology. To evaluate the effects of α-mangostin on mitochondrial morphology, we stained the mitochondria of C6 cells with MitoTracker™ Orange CMTMRos and the cell nuclei with DAPI dye. The results were observed under a fluorescence microscope (Fig. 6). Observations of the mitochondrial fluorescent staining images showed that in the untreated control sample, the mitochondrial fluorescence signal was very clear and widely distributed. However, when treated with α-mangostin at a concentration of 3 μg/ml, the mitochondrial fluorescence signal was slightly affected. At a concentration of 5 μg/ml, the mitochondrial fluorescence signal decreased further, and mitochondrial morphology shifted from an elongated phenotype to a more rounded form compared to the 3 μg/ml concentration. At a concentration of 8 μg/ml, the mitochondrial fluorescence signal significantly decreased, and the mitochondria reduced in size and exhibited a “fragmented” appearance.

Changes in mitochondrial morphology may be an adaptive response triggered by the loss of mitochondrial membrane potential. Therefore, to investigate the effects of α-mangostin on mitochondrial function, we conducted a study on the impact of α-mangostin on mitochondrial membrane potential (MMP). Measuring mitochondrial membrane potential in living cells is used to assess mitochondrial function. Thus, we evaluated whether α-mangostin affects MMP by using Rhodamine 6G fluorescence staining. Rhodamine 6G is a membrane-permeable cationic dye that stains mitochondria in living cells. Interestingly, the use of α-mangostin altered the mitochondrial membrane potential (ΔΨm) of C6 cells in a concentration-dependent manner (Fig. 7). The results of membrane potential measurements upon treatment with α-mangostin at various concentrations over 24 hours indicated a slight reduction in mitochondrial membrane potential at a concentration of 3 μg/mL, with a significant decrease observed at 5 μg/mL, reducing to 34.27 ± 1,24%, and further to 25.77 ± 0,98% at 8 μg/mL, compared to the control, with a significance value of $*P < 0.05$. Untreated control cells exhibited high fluorescence due to the accumulation of Rhodamine 6G in the mitochondria of healthy cells.

Discussion

Current therapies such as surgery, radiotherapy, and chemotherapy in the treatment of gliomas often have low therapeutic efficacy and are associated with numerous dangerous side effects. Therefore, developing a new treatment strategy that selectively targets cancer cells without causing harmful side effects is of great importance. With its abundant and diverse medicinal resources, Vietnam is one of the Southeast Asian countries with significant potential in medicinal plants, providing an advantage for research and discovery of active compounds for cancer treatment and improving human life. Mangosteen, a fruit primarily grown in Vietnam, Thailand, and other tropical countries, has a rind traditionally used as a natural remedy for dysentery, ulcers, and wound infections. Among the bioactive compounds isolated from mangosteen, xanthone derivatives, predominantly including α-mangostin, β-mangostin, and γ-mangostin, have been widely reported to exhibit anticancer biological activities in various cancers such as liver cancer, lung cancer, and breast cancer [16–18]. It has been reported that α-mangostin exhibits antiproliferative effects in colorectal cancer [19] and lung cancer [17]. Therefore, in this study, we demonstrated that the main component of Garcinia mangostana L. extract, α-mangostin affects C6 glioma cells.

In this study, we conducted an MTS assay to confirm the inhibitory effect of α- mangostin on cell viability. The results indicated that C6 cells treated with α-mangostin exhibited a concentration-dependent decrease in viability. After 72 hours of treatment, the IC_{50} value was 4.67 ± 0.199 μg/mL, demonstrating that α-mangostin possesses cytotoxic properties against C6 glioma cells. Janhom et al. demonstrated that α-mangostin effectively inhibits the proliferation of SH-SY5Y neuroblastoma cells in a dose-dependent manner. Treatment with 10 μM α-mangostin for 24 hours significantly reduced cell viability compared to control samples. Moreover, cell viability was reduced by more than 50% at concentrations of 20 and 40 μM α-mangostin [20]. According to the findings reported by Kaigiang Li et al. in 2020, another xanthone derivative from mangosteen rind, β-mangostin, showed inhibitory effects on C6 glioma cells with an IC_{50} of approximately 4.811 μM (Li et al., 2020). The results of these studies are consistent with our findings, where α-mangostin reduced the viability of C6 cells in a concentration-dependent manner, indicating that α-mangostin may be an effective agent in inhibiting cancer cell proliferation.

Next, we investigated the effect of α-mangostin on the nuclear DNA of C6 cells using a single-cell gel electrophoresis assay. The results showed that this compound caused damage to nuclear DNA, leading to DNA fragmentation and unwinding, forming DNA tails with a tail DNA percentage of 17.95 ± 6.277%. This indicates that α-mangostin partially impacts the DNA of the cells, causing breaks and tail formation. A chemotherapeutic agent currently used for the treatment of glioblastoma, such as TMZ, has been demonstrated to induce DNA damage and inhibit the cell cycle. In a study involving the neuroblastoma cell line SH-SY5Y, TMZ treatment was shown to cause significant DNA damage, as evidenced by the comet assay. The results indicated that the percentage of DNA in the tail increased with higher concentrations of Temozolomide, reflecting a corresponding increase in the extent of DNA [21]. These studies indicate that some current cancer treatment drugs also operate by impacting the nuclear DNA in cancer cells.

Mitochondria are the main energy source of cells and play a role in cell proliferation, growth, and differentiation through continuous fusion and fission processes [22]. Additionally, mitochondrial morphology plays a crucial role in regulating mitochondrial and cellular function. To assess the impact of α-mangostin on the mitochondrial morphology of C6 cells, we conducted mitochondrial staining with MitoTracker and nuclear staining with DAPI, followed by microscopy observation. The results showed that mitochondrial morphology changes in a concentration-dependent manner with α-mangostin; as the concentration of α-mangostin increases, mitochondrial size decreases and they adopt a 'fragmented' shape. This change appears to be an adaptive response due to altered membrane potential. Mitochondrial membrane potential (ΔΨm) is a determinant of ATP production functionality in mitochondria, and a decrease in membrane potential is indicative of cells losing energy supply and entering programmed cell death. Mitochondria are key regulators of cell death through changes in ΔΨm in response to various adverse conditions. Loss of mitochondrial membrane potential is one of the causes of inhibited proliferation and apoptosis in C6 cells when treated with the compound. Our study results indicated that ΔΨm in C6 cells decreased after 24 hours of treatment with α-mangostin. Higher concentrations of α-mangostin resulted in greater reductions in mitochondrial membrane potential, as

evidenced by the decreasing fluorescence intensity. The 2020 report by Li et al. demonstrated that β-mangostin (another xanthone extracted from the pericarp of mangosteen fruit) induces mitochondrial damage in C6 cells after 24 hours of treatment [23]. In a study evaluating the effectiveness of Temozolomide /Simva/ASH combination on glioblastoma cell lines U87MG and U251, it was found that these combinations influenced the cell membrane potential. The decrease in membrane potential, indicated by reduced fluorescence intensity, directly impairs mitochondrial function, diminishes ATP production, and induces oxidative stress. The reduction in mitochondrial membrane potential is a crucial step in the pathway leading to apoptosis [24]. Temozolomide activates the apoptosis pathway by causing DNA damage and impairing mitochondrial function. Synthesizing findings from previous studies and our research results, α-mangostin, a xanthone, was identified as capable of inducing cell toxicity, causing DNA fragmentation, and affecting the morphology and ΔΨm of C6 glioma cells. These effects contribute to its efficacy in treating glioblastoma through various cellular pathways, suggesting its potential for development as a chemotherapeutic agent for glioblastoma.

Conclusion

In this study, α-mangostin, a compound extracted from the rind of mangosteen fruit, has been demonstrated to exhibit potent antioxidant activity and the ability to inhibit cell proliferation, along with cytotoxic effects on C6 cells. The research findings indicate that α-mangostin induces DNA damage in cells after 24 hours of treatment. Observations of the morphology and membrane potential of C6 glioma cells also reveal significant alterations across different concentrations within 24 hours. These results provide compelling signals regarding the potential application of α-mangostin in the research and treatment of glioblastoma. This marks a significant advancement in exploring and developing new pharmaceuticals from natural plant-derived compounds, offering hope for more advanced therapeutic approaches for glioblastoma treatment.

Methods

Material

α-mangostin of purity was purchased from Sigma Aldrich.. The C6 mouse glioma cell line (American Type Culture Collection) was supplied by the Institute of Biotechnology - Vietnam Academy of Science and Technology. All chemicals and equipment used in this study were purchased from well-known suppliers such as Thermo Fisher, Gibco, Promega, etc.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the extract was determined following the method of Blois [25] with some modifications. The samples were dissolved in ethanol and ascorbic acid was used as a positive control. A 190 µL aliquot of 0.2 mM DPPH in ethanol was mixed with 10 µL of the extract at various concentrations (2, 10, 20, 50, 100 µg/mL). After a 30-minute incubation at room temperature, the

absorbance was measured at a wavelength of 517 nm. The radical scavenging capability was expressed as IC_{50}.

Cell Culture and Proliferation

The C6 mouse glioma cells were cultured in high glucose DMEM medium (Pan - Biotech, P04-05550), supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin under standard conditions (37℃, 5% CO2).

Cytotoxicity Assay (MTS assay)

The cytotoxic activity of α-mangostin was determined by using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS). Cells were cultured in a 96-well plate at a density of 5000 cells/well using a

complete medium. After 24 hours of incubation, the cells were treated with α-mangostin at various concentrations (0, 0.5, 5, 10, 15, and 25 µg/mL) and incubated at 37°C for 72 hours. Cell proliferation and viability were assessed using the MTS cytotoxicity assay kit ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). The MTS reagent was added to the treated C6 cell wells and incubated for 3 hours at 37°C. The PMS (phenazine methosulfate) present in the reagent is metabolized by the cells to produce a formazan product that is soluble in cell culture medium, with maximal absorption at a wavelength of 490 nm (measured using a Thermo Science™ Multiskan™ GO spectrophotometer) [26]. The IC_{50} value (the concentration at which the test compound is capable of causing 50% cell death) is calculated using the following formula.

Comet assay

C6 cells were treated with α-mangostin at various concentrations and with H_2O_2 (500 µM) as a positive control for 24 hours. To assess DNA damage, after treatment, cells were collected post-treatment and resuspended in 1% agarose gel before being transferred onto a glass slide pre-coated with agarose. The cells were lysed in a lysis solution (2.5M NaCl, 10 mM Tris HCl, 100 mM EDTA, 200 mM NaOH, pH 10) overnight at 4°C. After lysis, the slides were immersed in an alkaline solution (300 mM NaOH, 1 mM EDTA, pH > 13) for 1 hour at 4°C, protected from light. The slides were then subjected to cold electrophoresis in 1X TBE buffer for 30 minutes (1 V/cm) and subsequently rinsed with deionized water to neutralize the pH [27]. The DNA in the cell samples was stained with DAPI (1 µg/mL) and observed at 50X magnification using a Nikon Ti E Eclipse fluorescence microscope equipped with a confocal system to evaluate DNA fragmentation. The results were analyzed using CometScore 2.0 software.

Mitochondrial fluorescence staining

To evaluate the effect of α-mangostin on cellular mitochondria, C6 cells were treated with α-mangostin at concentrations of 0, 1, 3, 5, and 8 µg/ and then fixed in 4% paraformaldehyde for 10 minutes. Following fixation, C6 cells were stained with of mitochondria and nuclei were observed using a Nikon Ti E Eclipse fluorescence microscope, and the images were analyzed using ImageJ software [28]. MitoTracker™

Orange CMTMRos (1 mM) at 37°C for 30 minutes. The cell nuclei were stained with DAPI (1 µg/mL) for 30 minutes at room temperature. The samples were washed three times with 1X PBS. Fluorescent images of mitochondria and nuclei were observed using a Nikon Ti E Eclipse fluorescence microscope, and the images were analyzed using ImageJ software [28].

Mitochondrial Membrane Potential Assessment

Mitochondrial membrane potential was analyzed using the fluorescent dye Rhodamine 6G [29]. C6 cells were cultured in a 96-well plate at a density of 5000 cells/well and treated with α-mangostin at concentrations of 0, 1, 3, 5, and 8 µg/mL. After 24 hours, the C6 cells were stained with 20 µM Rhodamine 6G at 37°C for 60 minutes. The samples were washed three times with 1X PBS, and fluorescence intensity (excitation at 525 nm, emission at 595 nm) was detected using a SpectraMax iD5 Multi-Mode Microplate Reader.

Statistical Analysis

The data was evaluated by using the t-student test, and differences between samples were considered statistically significant at $p < 0.05$.

Declarations

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The authors deny any financial support in the production of research or publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of this manuscript and all associated data.

Author Contribution

HDT and ANK performed the experiments. NHN, ATMD, LTHN, TVN, LKN, and TTBL interpreted the data and provided scientific and statistical analysis. HDT and ANK prepared and wrote the manuscript. DTTL established the study design, provided the research strategy, and supervised the overall research plan. Funding was obtained by DTTL. All authors read and approved the final manuscript.

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Figures

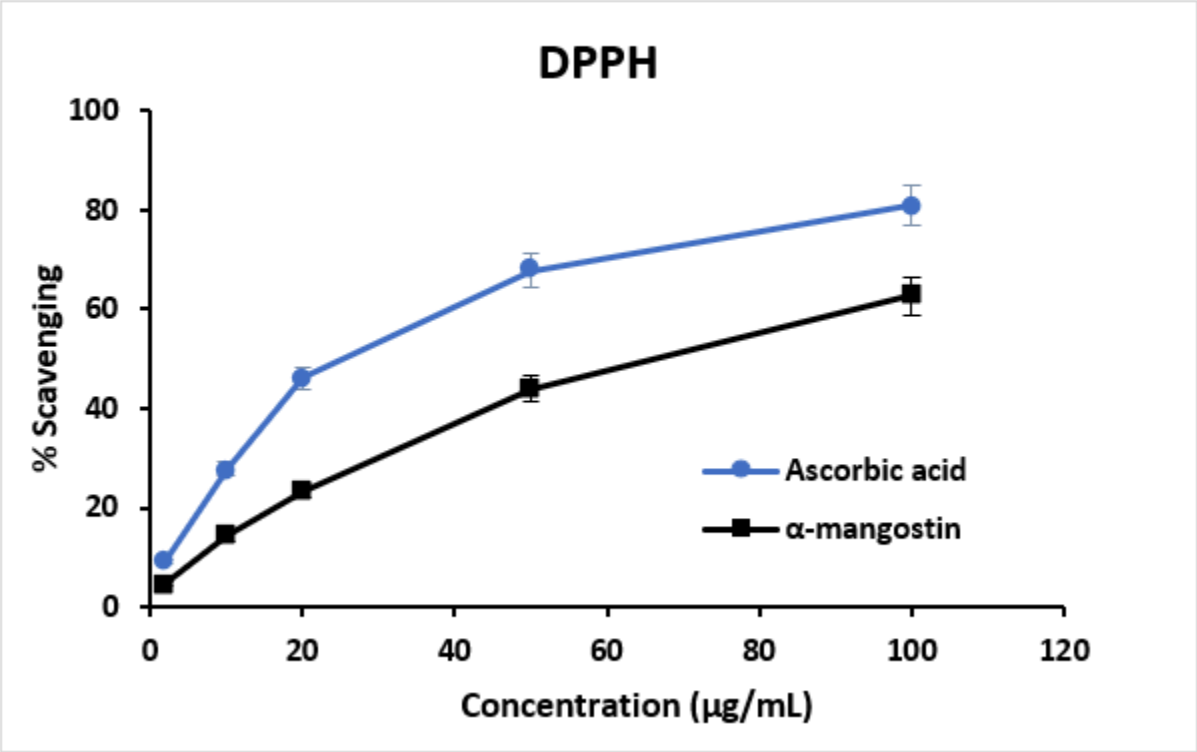


Figure 1

Scavenging effects of α-mangostin and ascorbic acid on DPPH radical

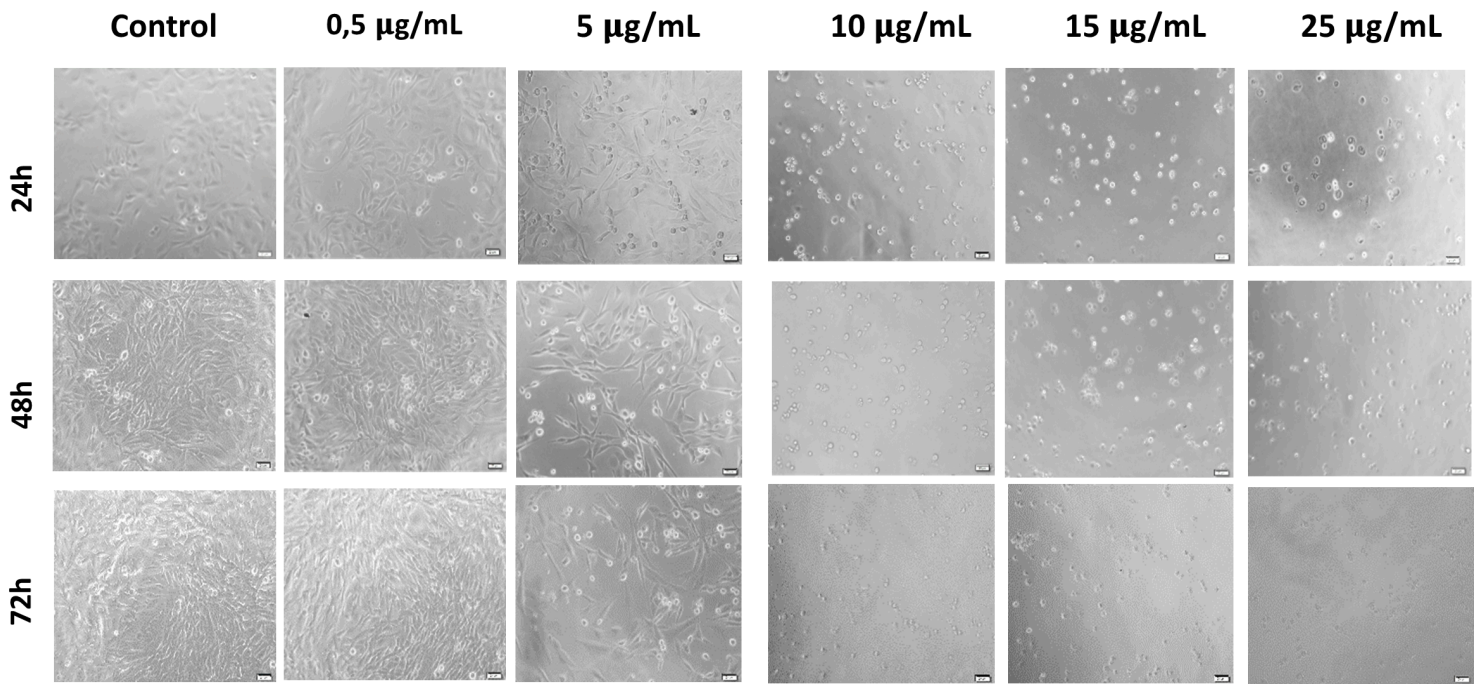


Figure 2

Effects of α-mangostin on C6 cells at various concentrations.

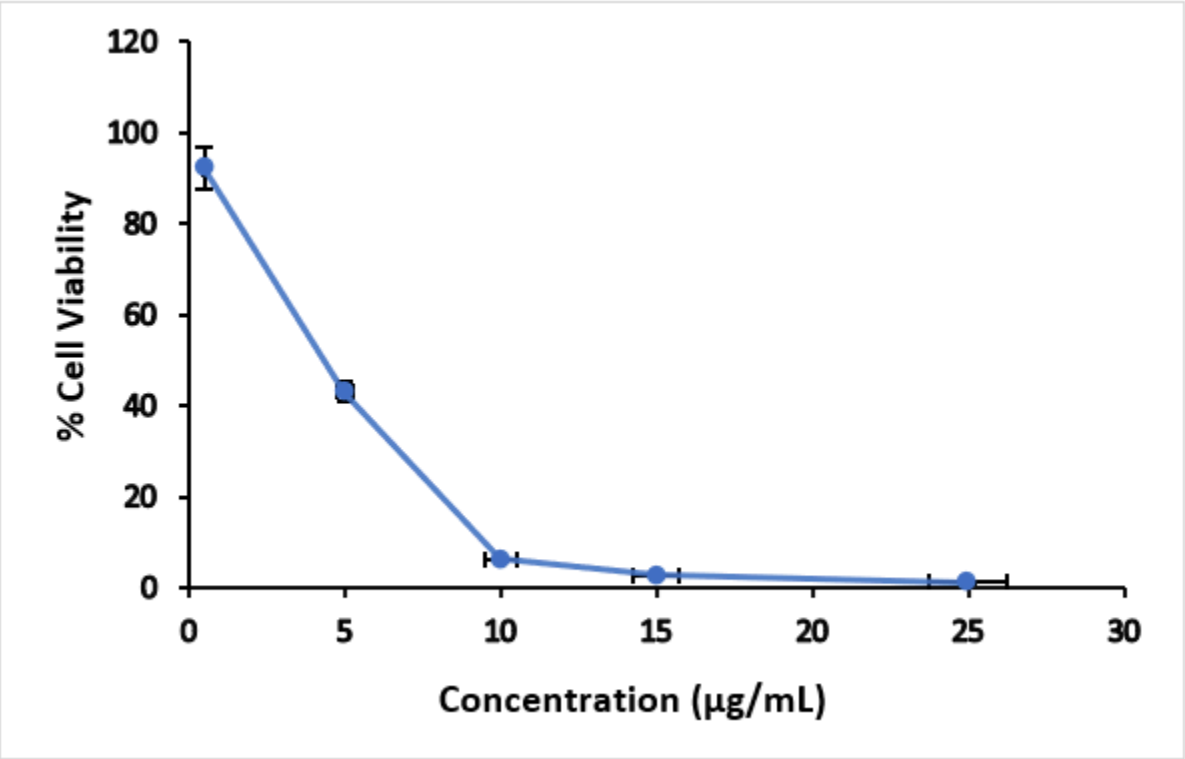


Figure 3

Effect of α-mangostin on cytotoxicity in C6 cells

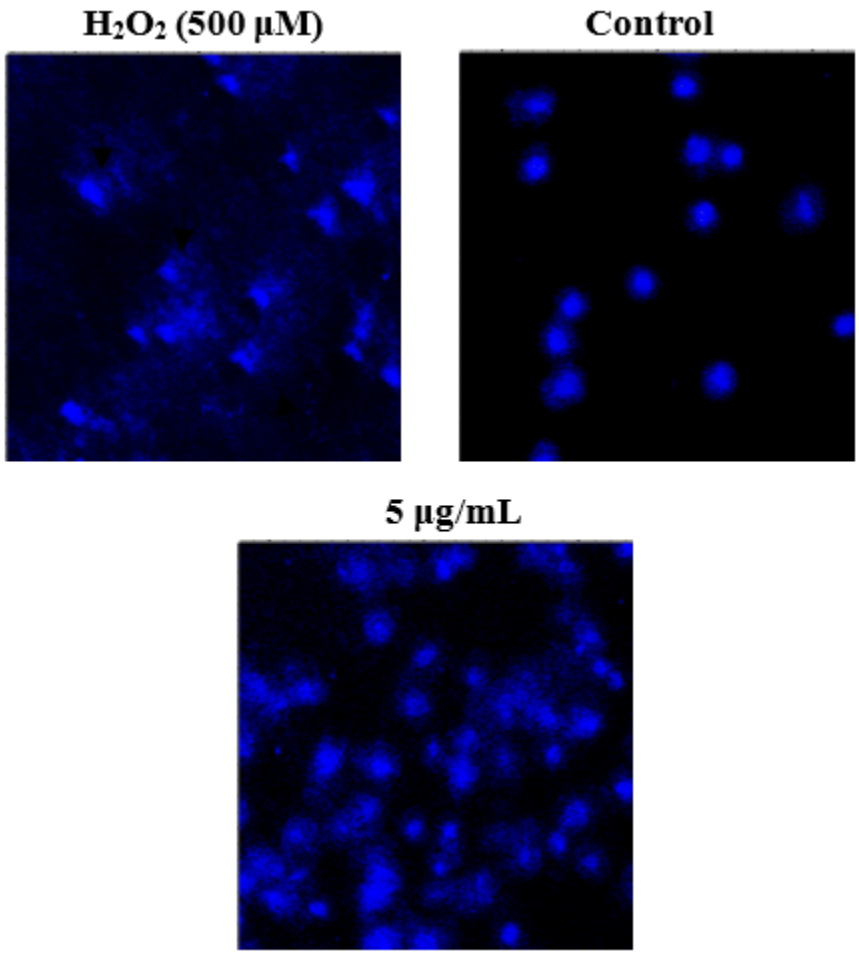


Figure 4

Results of single-cell gel electrophoresis

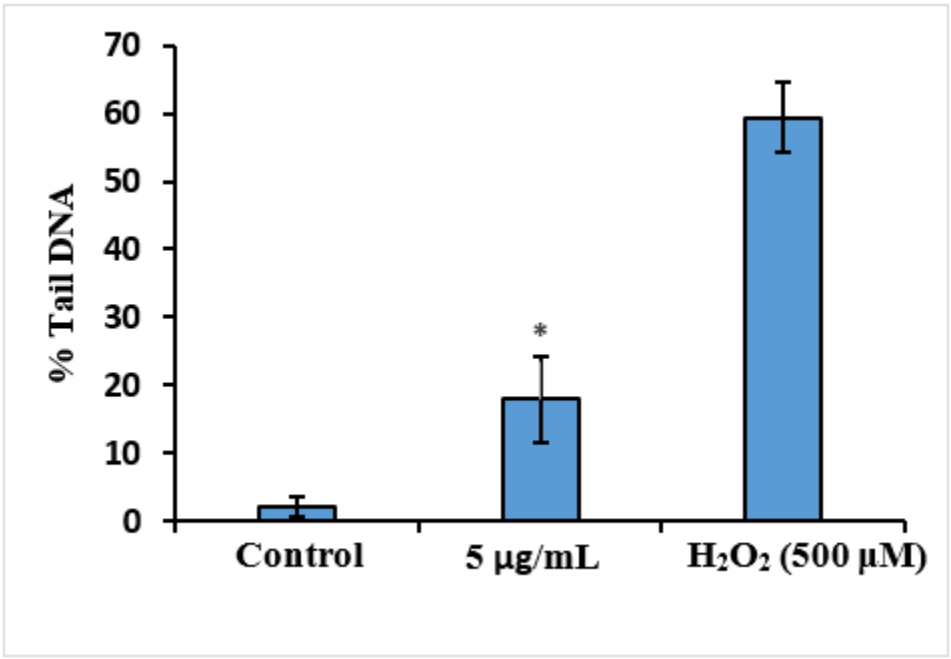


Figure 5

% Tail DNA at different α-mangostin concentrations (*P< 0.05)

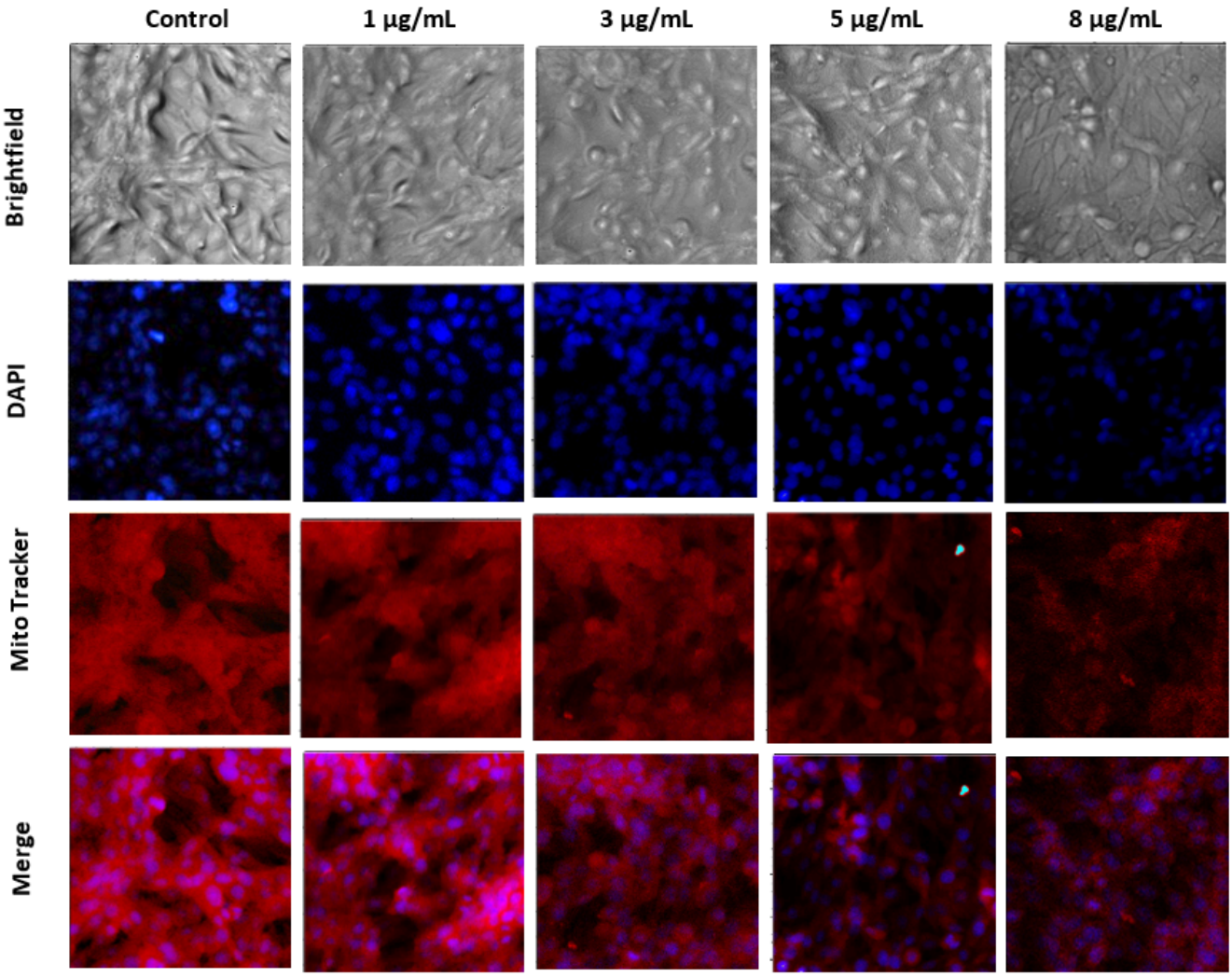


Figure 6

Effect of α-mangostin on mitochondrial morphology in C6 cells

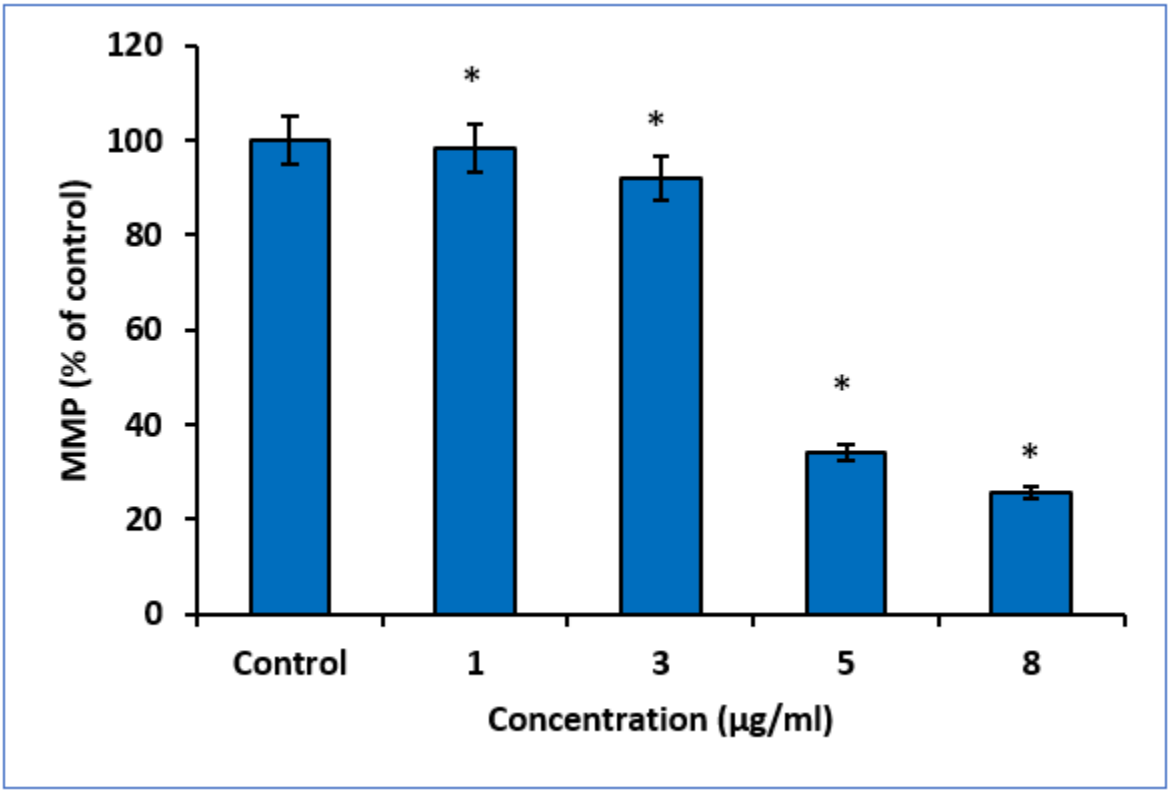


Figure 7

Effect of α-mangostin on mitochondrial membrane potential in C6 cells (*P < 0,05)