Original Research Article

α-solanine suppresses glioblastoma multiforme growth and metastatic properties by modulating the apoptosis-autophagy axis

Abstract

Introduction

Glioblastoma multiforme (GBM) has a poor prognosis despite optimal treatment. Recent studies have shown the potential of phytochemicals as anti-cancer agents. α-solanine, derived from plants of the Solanum genus, is a promising molecule in this regard. This study investigated the efficacy of α-solanine compared to temozolomide (TMZ) against GBM cell lines (U87MG, U251, and T98G) in vitro.

Methods

In-vitro assays were conducted to assess the viability, migration, invasion, and mode of cell death of U87MG, U251 and T98G GBM cell lines following α-solanine treatment in comparison to TMZ. Rt-qPCR and proteome profiling were conducted to investigate the changes induced by α-solanine on a molecular level.

Results

α-solanine demonstrated potent cytotoxicity on all GBM lines, with IC_{50} values ranging from 19.66 µM to 22.87 µM, and significantly inhibited GBM cell migration compared to TMZ treatment. RNA and protein level assays indicated upregulation of both apoptotic and autophagy proteins, suggesting the involvement of BECN1 and BNIP3L in α-solanine-induced cell death.

Discussion and conclusion

These findings contribute to the search for effective GBM treatments. Future studies should increase the number of biological replicates, employ alternative methods to strengthen the findings, and conduct in vivo experiments and testing using patient-derived GBM tissue to better evaluate any therapeutic suitability of and fully understand the mode of action of α-solanine on GBM.

Key words: α-Solanine suppressed, glioblastoma, anti-cancer agents

Introduction

Glioblastoma multiforme (GBM) is the most commonly occurring malignant brain tumour, representing approximately 50 % of all primary malignant central nervous system tumours (CNSTs) [1]. GBM is classified as a grade-IV glioma, the highest grade afforded by the World Health Organisation (WHO) classification [2]. Routine treatment of GBM involves maximum surgical resection (MSR) of the tumour with the administration of adjuvant temozolomide (TMZ) being established as the first-line chemotherapeutic treatment for histologically confirmed GBM [3]. Despite optimal treatment, the prognosis of GBM is still abysmal, with the median survival being estimated at 15 months [4].

Thus, GBM is not only a major healthcare burden, but also a socio-economic burden that requires costly treatment and has minimal improvement in patient prognosis [4,5]. This emphasizes the necessity and urgency to identify novel and cost-effective putative therapeutic strategies for treating GBM [6].

Recent research on alternative anti-cancer therapeutic approaches has focused on bioactive compounds derived from natural sources such as plants and microorganisms [7,8]. A growing body of literature has recently demonstrated the effectiveness of ∝-solanine, a steroidal glycoalkaloid that can be derived from *Solanum* plants, as a putative chemotherapeutic anti-cancer drug [9].

∝-solanine's anti-cancer potential has been extensively studied against several different cancers and this phytochemical is thought to inhibit cancer cell growth, proliferation and metastasis primarily by modulating Akt/MTOR and Ras/Rak/ERK pathways as well as MMP expression. Moreover ∝-solanine has been documented to induce both apoptotic and autophagic death, however the exact mode of cell death induced by ∝-solanine is still disputed.

Apoptosis and macro-autophagy are the two main types of cell death [9,10]. In glioblastoma multiforme (GBM), apoptosis deregulation is an intrinsic hallmark, caused by an imbalance in anti- and pro-apoptotic proteins [11–15]. Chemotherapy and intra-tumoral hypoxia can increase baseline autophagy, leading to increased tumoural growth and metastatic potential [16].

Apoptosis and autophagy share regulatory machinery [17–19]. The BECN1/Bcl-2 complex inhibits autophagy in nutrient-rich conditions while favouring autophagy during starvation [20,21]. Initiator and effector caspases inhibit autophagy by degrading Atg3, Atg5, and BECN1, preventing the assembly of autophagy machinery leading to apoptosis. Atg5 acts as a signaling regulator between autophagy and apoptosis by interacting with Atg12 and Bcl-xL. Atg12 induces mitochondrial apoptosis by inhibiting Bcl-2, leading to Bax activation and cytochrome-c release [22].

Increased anti-apoptotic proteins and autophagy are associated with worse patient prognosis, resistance to TMZ treatment [23], and increased recurrence [16,24]. Thus, modulating cell death is a promising therapeutic strategy for GBM [25].

Phytochemicals have emerged as promising modulators of the autophagy-apoptosis pathway [26], with promising potential against GBM [27]. Glycoalkaloids are increasingly being proven as promising anticarcinogenic agents [28]. α-solanine, the most abundant glycoalkaloid, has demonstrated anticarcinogenic effects, yet the precise mechanisms leading to cell death are not yet clear [9,10].

In this study, we investigate the effects of α-solanine on the viability, metastatic potential, and cell-death signaling in comparison to TMZ treatment, while explicating the cell-death mechanisms induced by α-solanine. We hypothesize that α-solanine exerts its anti-cancer effects by altering the autophagy-apoptosis axis, rather than directing cell death through apoptosis or autophagy.

Methods

In-vitro assays were conducted to assess the anti-cancer effects of α-solanine's on U87MG, U251 and T98G cell lines.

Reagents and materials

>95% pure α-solanine (Santa Cruz Biotech, 20562-02-1) and TMZ (Cayman Chemical company, 85622-93-1) stock solutions (5mM and 8mM, respectively) were stored at -80 ^{o}C until preparation and subsequent dilution to the desired working concentration, before use. EDTA, Trypsin, PBS, FBS, and DMEM/F12 were purchased from Sigma Aldrich (St Louis, Missouri, USA).

Cell culture and drug treatment

U87MG cancer cell line was obtained from the Centre for Molecular Medicine and Biobanking at the University of Malta. U251 and T98G cell lines were obtained from CLS GmbH (Eppelheim, Germany). These cells were chosen since they have similar gene expression and morphological characteristics to patient tumors. Moreover, T98G cells are resistant to TMZ, while U251 and U87MG are known to be sensitive, but still demonstrate varying degrees of resistance. Cells were cultured in DMEM/F12 medium with 10% human serum, 1% penicillin/streptomycin, 1% amphotericin, 0.2mg/ml platelet lysate and 50 µg/mL of heparin in 37 °C, 5% CO_2 and > 95% humidity incubator. When cells were at approximately 80% confluency, trypsin/EDTA buffer was used to detach the cells for subculture or for corresponding experimental treatments. An inverted microscope was used to observe the changes in cell morphology. α-solanine and TMZ were added to cell culture medium after a confluency of >80% was obtained during each respective experiment, as outlined below. 5.00 mM H_20_2 was used as a positive control throughout the experiments since α-solanine's primary mode of action is the generation of reactive oxygen species.

Viability assay

PrestoBlue^{TM} cell viability reagent (Invitrogen) was used to detect whether different concentrations of ∝-solanine (5, 25, 60, 75 µM) and TMZ (800, 1200, 1600, 2000, 2400 µM) could inhibit the survival of GBM cells. A density of 1 x 10^4 cells was plated in 96-well plates, and cells at 80% confluence were treated with different concentrations of ∝-solanine or TMZ, positive (5.00 mM H_2O_2) or negative (serum-free DMEM/12) control. After 72 hours of treatment, the medium was changed and 10 µl of PrestoBlue^{TM} was added to all the microwells. Using a Mithras LB 940 multimode microplate and MicroWin 2000 software

colorimetric readings were taking 2 hours after adding the PrestoBlue™ reagent, at 490 nm. Each experiment was conducted in biological triplicate.

Migration and invasion assays

Migration and invasion of the different treatment groups were verified by scratch migration and trans-well invasion assays respectively. After detaching, GBM cells were seeded in scratch assay inserts (ibidi) in a 24-well plate. After 24 hours the inserts were removed, and treatment conditions were added. Cell migration was monitored at 0, 24, 48 and 72 hours following the addition of treatments using an inverted light microscope. Migration distance was calculated using ImageJ (Fiji) software wound healing add-on [29]. Relative wound closure (%) and rate of relative wound closure (%) were respectively calculated using equation 1 and equation 2. Each experiment was conducted in biological triplicate.

$$Relativewoundclosure = \frac{Treatmentwoundarea(B) - TreatmentWoundarea(A)}{Meancontrolwoundarea(B) - meancontrolwoundarea(A)}$$

Equation 1.: Calculating the relative wound closure.

$$= \frac{Rateofrelativewoundclosure}{\frac{Relativewoundclosure(72hours) - relativewoundclosure(24hours)}{72 - 24}}$$

Equation 2.: Calculating the rate of relative wound closure

Trans-well inserts with an 8 µm pore size were placed in a 24-microwell plate. GBM cells at a confluency of $1x10^4$ cells were seeded in individual inserts. The microwells were filled with media containing 2.00% serum and different substances were added. After three days of incubation, images of five fields of view per microwell were taken using a Nikon Eclipse Ti Inverted microscope, and the number of cells that invaded through the microwells was recorded. Each experiment was conducted in biological triplicate.

Reverse transcription, Rt-qPCR and Sanger sequencing

Total RNA was obtained from GBM cell lines (cultured to a confluency of $1x10^4$) using GeneJET RNA Purification kit (Thermo Scientific) using supplier's instructions. Reverse transcription was conducted using iScript reverse transcription supermix (Bio-Rad) according to supplier's instructions. Gene expression level was determined through rt-qPCR using the Applied Biosystems™ SYBR™ Green PCR Master Mix (Thermo Scientific) on a RotorGene Q (Qiagen, Netherlands; and in-house designed RNA primers. All primers were validated bioinformatically using Primer-BLAST, obtained from IDT Europe and validated experimentally through gel-electrophoresis visualisation of end-point PCR. Primer sequences were as follows:

ATG4B – F: GGTGTGGACATGATCTTGCC, R: CTCCACGTATCGAAGACAGC; Bax – F: GGACAGTAACATGGAGCTGC, R – GAGGAAGTCCAATGTCCAGC; BECN1 – F: GAGTTTCAAGATCCTGGACC, R – CTCCCCAATCAGAGTGAAGC; Casp-3 – F: GAGAACCACTGAAAACTCAGTGG, R – GAATGTTTCCCTGAGGTTTGC; IDH1 – F: AATCAGTGGCGGTTCTGTGG, R: CAAGTAGTCAGAACGTTGC. The RPLP0 [F: CTCTGCATTCTCGCTTCC; R: TGGCTTCAACCTTAGCTGG] housekeeping gene was used since this

was identified as being the most stable throughout the treatments. Rt-qPCR data was analysed by using the ΔΔCt method. Each experiment was conducted in biological duplicate. Sanger sequencing against the IDH gene using the in-house designed primers was performed by MLS BioDNA Ltd.

Protein extraction, proteome profiling and protein-protein interaction network analysis

The cell lysis buffer and protein extraction reagents provided with the antibody array profiling kits for apoptosis and autophagy (RayBioTech) were used to extract proteins (2mg/ml, diluted ten-fold when incubating the membranes with the extracted proteins) from treated GBM cells (cultured to a confluency of $1x10^4$) according to the manufacturer's instructions. Protein concentrations were quantified using Bradford assay and proteome profiling using the membranes provided with the antibody array kits and IRDye® anti-rabbit and anti-mouse IgG secondary antibodies (Li-Cor® Biosciences) according to supplier's instructions. Resultant dot blots were detected using an Odyssey infrared imaging system (Li-Cor® Biosciences) and densitometry analysis was conducted using ImageJ (Fuji). Fold change and protein-ratio *p*-value were used to assess whether proteins were significantly differentially expressed between treatment and control conditions, with a fold change value of $\leq$0.7 / 1.5$\leq$ (+/- 0.05) being set at the cut-off value for down-regulation and upregulation respectively.

The top 5 predicted direct protein-protein interactions, which have an apoptotic or autophagic biological function, for the proteins assayed in the apoptosis and autophagy antibody array kits were identified using UniProt, GeneCards and String v10.5 databases. The interactions used in this study were obtained from reliable sources, including high-throughput experiments and curated databases, ensuring a high level of confidence. Cytoscape software (version 3.9.1) was used to construct the protein-protein interaction network (PPIN), which was presented as a perfuse force directed layout. The PPI network was then pruned to only demonstrate significantly differentially expressed proteins identified through antibody array assays. Significantly up or down regulated proteins were presented by colouring 'nodes' in the form of a heat map.

Statistical analysis

All analyses were performed using JASP version 0.15. The results were expressed as the means and S.E.M. of multiple experiments or representative images. T-testing or ANOVA was utilised to distinguish the differences between groups. Three levels of $\propto$-value were determined for better graphical representation: (*) 0.05 $\geq$ p-value > 0.01; (**) 0.01 $\geq$ p-value > 0.001; (***) p-value $\leq$ 0.001.

Results

All cell lines are GBM $IDH_{wildtype}$

Following the WHO 2021 CNST classification, it has become essential to disclose the mutation status of the IDH1 gene to confirm GBM cell status. The IDH-1 gene is most frequently mutated at R132 (Alzial et al., 2022). End-point PCR and subsequent sanger sequencing performed for the IDH-1 gene for U87MG, U251 and T98G cell lines demonstrated the absence of mutations at R132 (Figure 1A). No additional mutations were

noted. Hence, the cell lines used in this study can be classified as WHO grade IV GBM $IDH_{wildtype}$.

Glioblastoma cells are more sensitive to α-solanine than to Temozolomide

The viability of U87MG, U251 and T98G cells against different concentrations of α-solanine and TMZ was investigated. Figure 1B indicates that all 3 cell lines demonstrated resistance against TMZ treatment, with T98G cells being the most resistant. In contrast, α-solanine had a significant dose-dependent cytotoxic effect on U87MG, U252 and T98G cells (Figure 1C). α-solanine IC_{50} data for each cell line was generated, and are represented in Figure 1D. TMZ treatment did not achieve IC_{50} experimentally and this data could not be extrapolated graphically due to the resistance against TMZ which occurred. Under normal culture (negative control) conditions, all three GBM cell lines exhibited stellate morphology, typical of glial cells. U87MG cells did not achieve the same culturing confluency as U251 and T98G cells. 5 mM H_2O_2 (positive control) treatment markedly changed GBM cell morphology, with all three cell lines losing their gross morphological characteristics with resultant characteristic necrotic appearance. Treatment with 1200 µM TMZ and 6.25 µM α-solanine respectively produced similar changes in GBM cell morphology, which suggested presence of cell stress (Figure 1E).

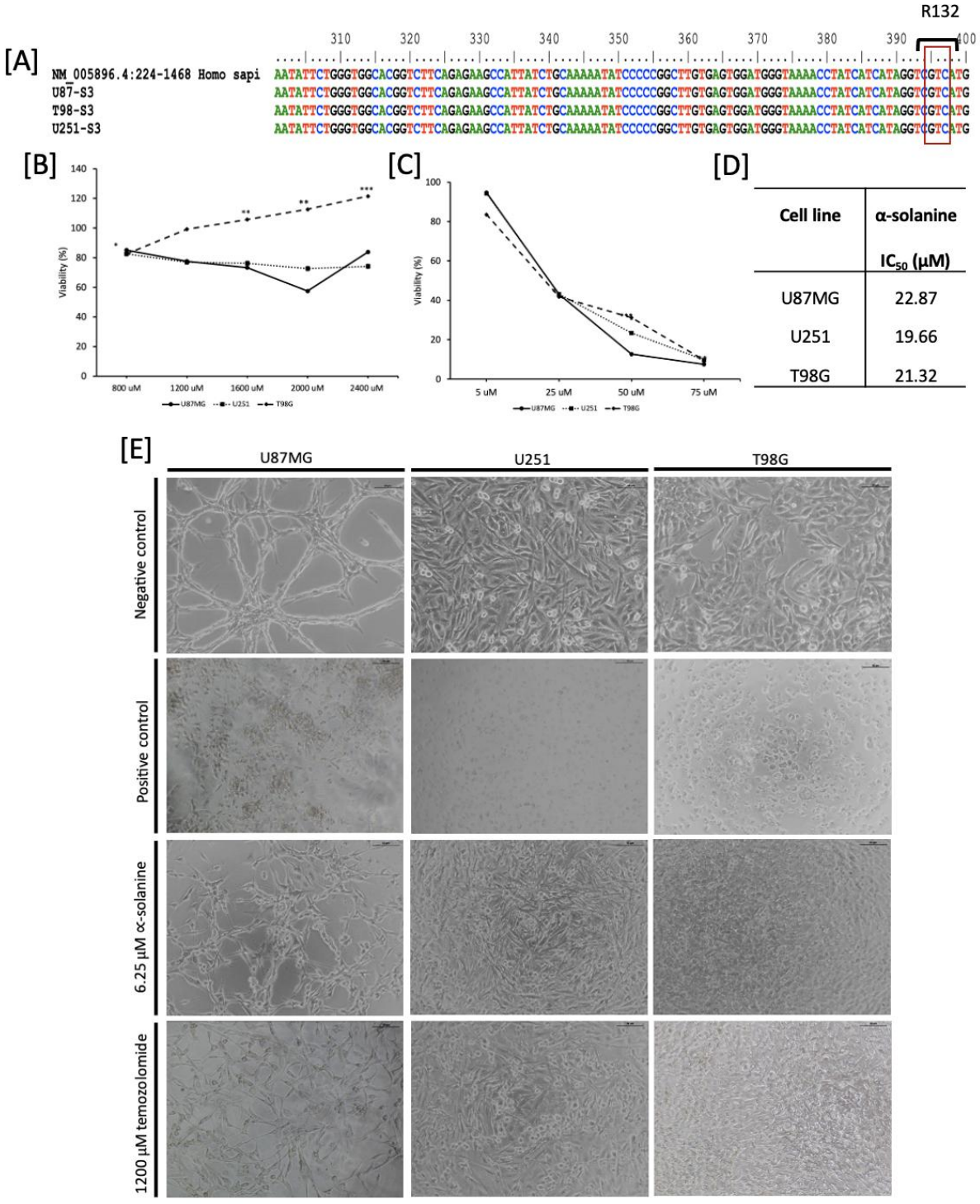


Figure 1: IDH1 gene sequencing and α-solanine's effect on GBM cell viability and morphology. [A] IDH1 gene sequencing; [B] TMZ viability assay; [C] α-solanine viability assay; [D] α-solanine IC_{50} data, and; [E] effect of negative control, positive control, α-solanine and TMZ treatment on GBM cell morphology (Magnification x100).

α-solanine demonstrated a greater reduction in GBM cell migration and invasion

Cell migration potential of U87MG, U251 and T98G cells under 1200 μM TMZ or 6.25 μM α-solanine treatment compared to negative control was investigated at 24, 48 and 72 hours using scratch migration assays. Visual inspection of serial imaging demonstrates a decrease

in wound area across time, across all cell lines and treatment conditions (Figure 2A). α-solanine treatment decreased relative wound closure across all cell-lines at each time point investigated. However, relative wound closure across all cell lines was observed to be significantly reduced after 24-hour incubation with 6.25 µM α-solanine in-comparison to 1200.00 µM TMZ treatment. Moreover, this observation was also noted in U87MG after 72-hours of treatment and U251 cells after 48-hours of treatment (Figure 2B, C, D). α-solanine significantly decreased the rate of U87MG cell migration in comparison to TMZ treatment, however this observation was not extended to the relative rate of U251 and T98G cell migration (Figure 2E).

Trans-well invasion assays were conducted to investigate the effect of α-solanine treatment in comparison to TMZ, on GBM cell invasion. 6.25 µM α-solanine significantly decreased U87MG and U251 but not T98G cell invasion in comparison to 1200 µM TMZ.

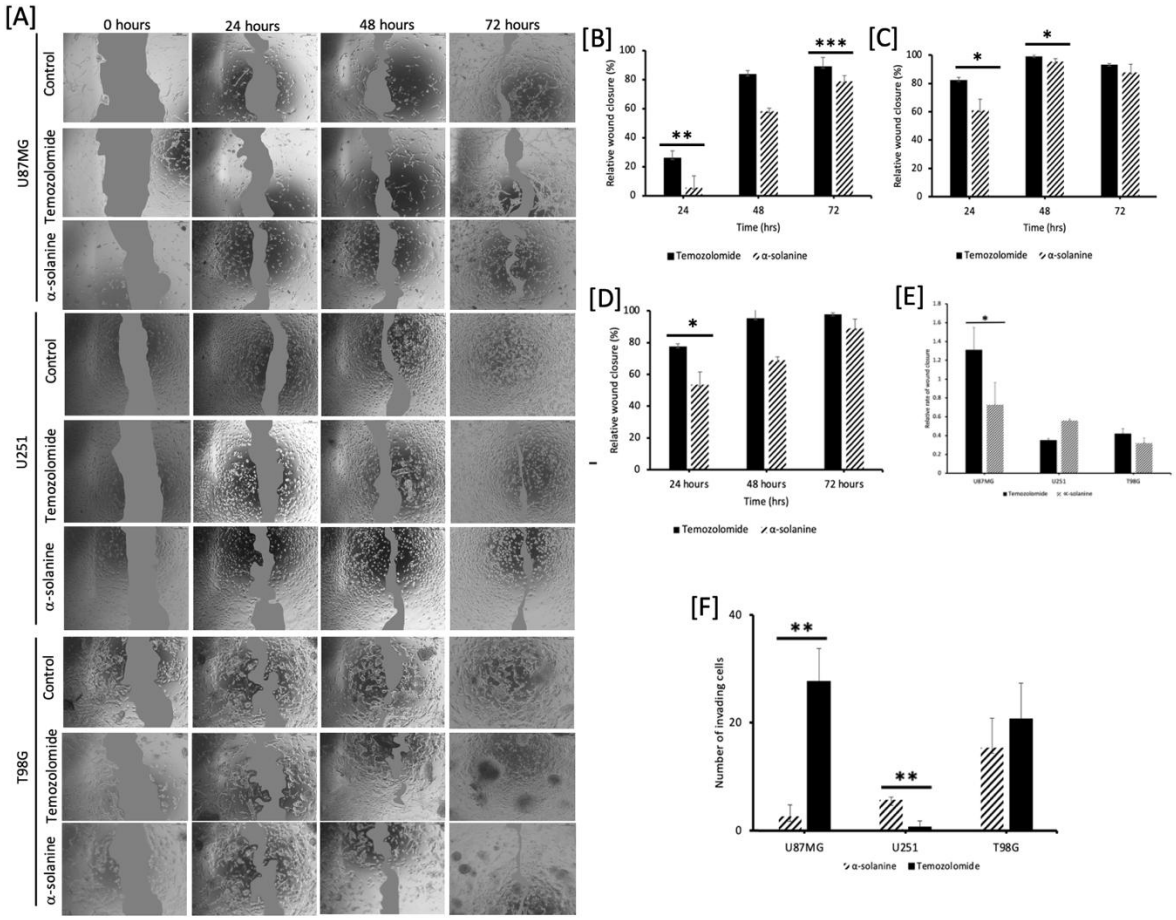


Figure 2: α-solanine decreases GBM cell migration and invasion. [A] Representative images of GBM cell migration at 0, 24, 48 and 72 hour time points (Magnification x40); effect of α-solanine treatment in comparison to TMZ on; relative wound closure in [B] U87MG; [C] U251; [D] T98G cells; [E] relative rate of GBM cell migration and [F] GBM cell invasion.

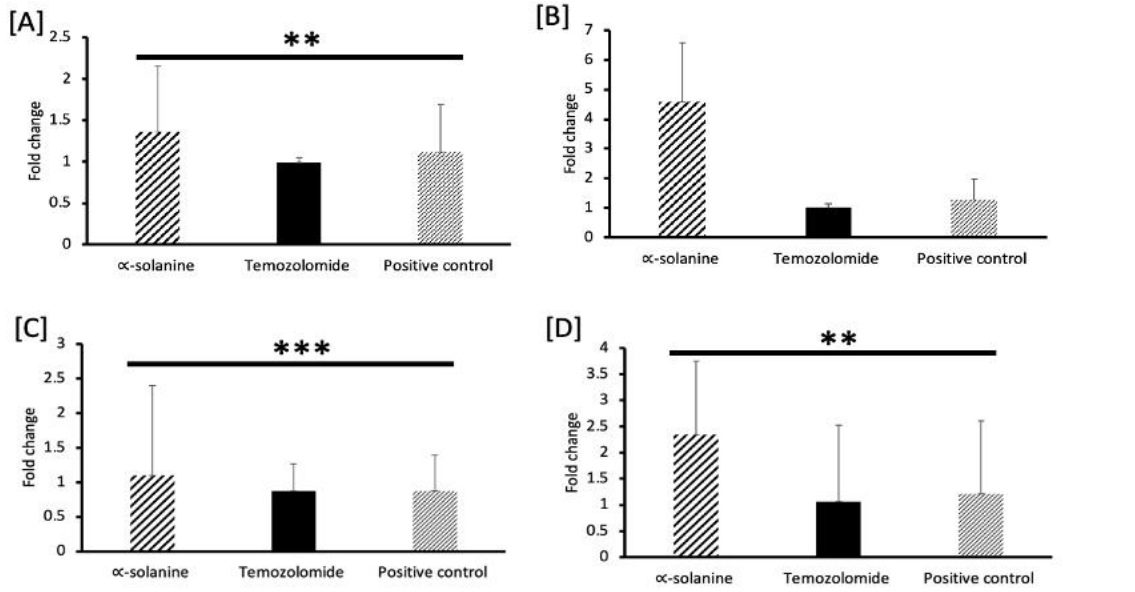
α-solanine induced increased expression of Bax, BECN1 and ATG4B genes.

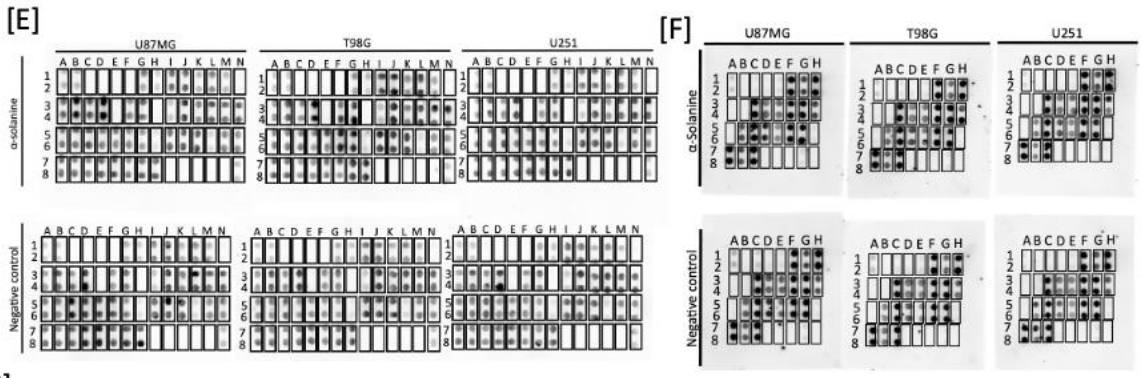
Rt-qPCR was conducted to assess the effect of 1200 µM temozolomide versus 6.25 µM α-solanine versus 5.00 mM H_2O_2 (positive control) on Bax, Casp-3, BECN1, and ATG4B gene

expression. These genes were chosen since these are often considered as vital regulators of the apoptotic and autophagic cell death. α-solanine treatment significantly increased the expression of Bax, BECN1, and ATG4B genes (Figure 3A-D).

Both apoptotic and autophagic proteins were upregulated following α-solanine treatment

Since rt-qPCR demonstrated that α-solanine significantly increased the expression of both apoptotic and autophagic genes, antibody arrays were used to investigate the effect of 24 hour 6.25 µM α-solanine treatment in comparison to negative control (culture) conditions on the expression levels of proteins involved in apoptosis and autophagy. Of the 63 investigated proteins, only 18 satisfied the predetermined criteria to be considered as differentially expressed, with all 18 proteins being upregulated (Figure 3G). PPI network analysis demonstrated that assayed proteins which were differentially expressed and interact with each other may be involved in the induction of autophagy and caspase independent apoptosis (Figure 3H).





[G]

Protein	Fold Change	Pair-wise sample testing
Bad	1.50	0.02
Bcl-2	1.54	<0.001
Bcl-w	1.47	<0.001
Bid	1.64	<0.001
Bim	1.74	0.02
TNFRSF5	1.57	0.02
TNFSF5	1.57	0.02
cIAP-2	1.48	0.01
Cyt-C	1.46	0.02
Fas	1.67	0.03
Fas ligand	1.73	0.04
IGF-2	1.47	0.03
IGF-1R	1.71	0.07
ATG4A	1.47	<0.001
ATG4B	1.46	<0.001
BECN1	1.46	<0.001
BNIP3L	1.54	<0.001
LC3A	1.46	<0.001

[H]

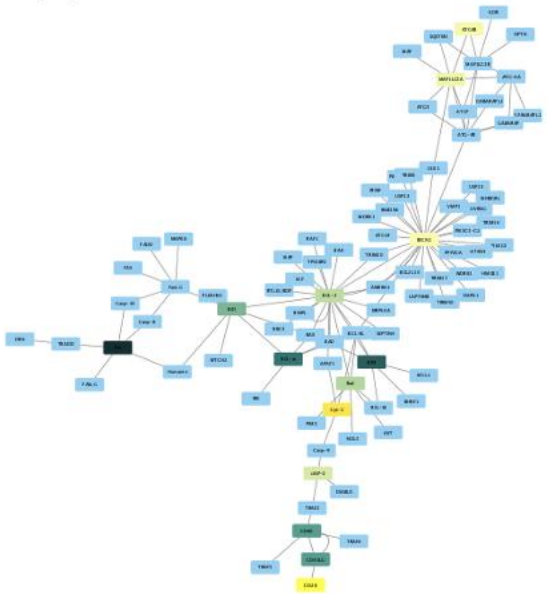


Figure 3: Effect of α-solanine on selected genes and apoptosis-autophagy proteins. Rt-qPCR results for [A] Bax; [B] Casp-3; [C] BECN1 and [D] ATG4B; profiling membranes dot blots for

[E] apoptosis and [F] autophagy proteins; [G] noted upregulated proteins, and [H] protein-protein interaction network analysis for the resultant upregulated proteins.

Discussion

Following the post-Stupp treatment regimen era for treating GBM [30], and the advent of the WHO CNST 2021 being implemented across the board [2,31], global efforts are being made to identify putative therapeutic agents which demonstrate superiority over TMZ against WHO grade IV $IDH_{wildtype}$ GBM. Phytochemicals have emerged as a promising, accessible, and abundant source of chemicals which may have potential chemotherapeutic significance against GBM [27]. α-solanine, a glycoalkaloids derived from *Solanum* plants such as potatoes and tomatoes [32], has emerged as a promising anti-cancer agent against a multitude of different neoplasms [10] .

In this study, we conducted the first assessment of α-solanine's cytotoxicity and its effects on the migration and invasion properties of GBM cells. We examined both TMZ-sensitive and -resistant cell-lines and compared the efficacy of α-solanine with that of TMZ. H_2O_2 was used as a positive control since current literature evidences that α-solanine primarily produces its cytotoxic effects by generating ROS. Culture conditions (DMEM/F12) was used both as vehicle for α-solanine and TMZ as well as the negative control. Moreover, this project strived to increase the current understanding of α-solanine's effect on the apoptosis-autophagy axis on a molecular level, by employing experimental and bioinformatic techniques.

In-light of the WHO 2021 CNST classification, the IDH gene of all three GBM cell-lines used throughout this project was sequenced since suppliers only specify that the cell lines use throughout this project were high-grade astrocytoma even through it is widely accepted that these cell lines may be considered as GBM. WHO grade IV $IDH_{wildtype}$ status was confirmed, and thus study provides insight on α-solanine's effect on true GBM.

Literature has demonstrated that α-solanine may potentially be an effective cytotoxic agent against numerous cancers, with documented 24-hour and 48-hour IC_{50} ranging between 10.00 µM – 32.18 µM and 9.65 µM – 20.84 µM respectively [11,15,33–35]. Yet published evidence against its efficacy against GBM is ambiguous. In this study the effect of α-solanine treatment was assessed against both TMZ-resistant (U87MG and U251 cell lines) and TMZ-sensitive GBM (T98G cell line), with the experimentally obtained IC_{50} being congruent with that reported in published literature, ranging from 19.66 µM – 22.87 µM. Yet, it is worth noting that in this project cell viability assays were conducted for 72-hours, as opposed to 24- and 48-hours reported in published literature assessing the viability of different cancer following α-solanine treatment. Seventy-two hour incubation with α-solanine was conducting instead of 24-hour or 48-hour since the GBM cells used in this study have long doubling times [36]. Through logistic regression of cell viability data obtained, the working concentration (IC_{25}) of α-solanine (6.25 µM) and TMZ (1200 µM) was deduced and used throughout the remainder of the project. IC_{25} was used since to ensure that any observed effects could not be attributed α-solanine's cytotoxic properties.

The efficacy of α-solanine against TMZ treatment on GBM cell lines was assessed during this study. TMZ treatment IC_{50} against all three cell lines used in this project was not achieved.

T98G cells demonstrated greater resistance to TMZ, which was expected since this cell line is known to be inherently resistant to TMZ. Yet, U87MG and U251 cells have been documented to also develop resistance to TMZ, which may explain the viability results obtained in this study [37].

α-Solanine treatment was more potent than TMZ treatment, however TMZ IC_{50} was not achieved experimentally in this study, even though concentrations up to 2400 µM were assessed against GBM cell viability. This constraint impeded the ability to perform inferential statistical comparisons between the treatment of α-solanine and TMZ. The inability of TMZ treatment of reach that IC_{50} threshold even at concentrations exceeding 100 times the concentration achieved by α-solanine implies that the α-solanine compound exhibits greater potency than TMZ against GBM cells in an *in-vitro* setting.

The effect of α-solanine on GBM cell metastatic properties were also assessed. Migration of GBM cells was decreased in a time dependent manner, however the effect of different doses on the GBM cell migration was not assessed. Our findings are congruent with published literature which demonstrated that α-solanine decreases choriocarcinoma, lung, hepatocellular colorectal, pancreatic, hepatocellular and oesophageal carcinoma cell migration [11,38–41]. Moreover, this study has also demonstrated that α-solanine reduces GBM cell migration and invasion in comparison to TMZ, suggesting increased potency in decreasing metastatic properties over TMZ *in-vitro*.

Select genes were assayed using rt-qPCR to determine whether α-solanine dysregulated their expression levels. Notably, Bax, BECN1 and ATG4B were significantly upregulated in comparison to TMZ and positive control treatment, as demonstrated by post-hoc testing. The upregulation of the aforementioned genes supports the hypothesis that α-solanine does not induce solely apoptosis or autophagy.

It is important to note that the number of genes investigated in this study was limited, representing a partial snapshot of the molecular response of GBM cells to α-solanine treatment. To complement the rt-qPCR analysis and gain a broader understanding of the molecular changes induced by α-solanine, proteome profiling of proteins involved in apoptosis and autophagy was conducted, since this approach enabled the identification and quantification of a larger set of proteins associated with the apoptotic and autophagic cellular response. Through employing rigours threshold values Bad, Bcl-2, Bcl-w, Bid, Bim, CD40, CD40 ligand, ciAP-2, Cyt-C, Fas, Fas ligand, IGF-2, IGF-1R, ATG4A, ATG4B, BECN1, BNIP3L and LC3A proteins were observed to be upregulated. These results are supported by Hasanain et al. (2015) who demonstrated the upregulation of proteins involved in autophagosome formation. Yet, numerous studies documented Bax upregulation and Bcl-2 down-regulation [12,44–49]. Rt-qPCR experiment supports bax upregulation, however proteome profiling demonstrated the opposite effect of Bax downregulation and Bcl-2 upregulation, further suggesting that α-solanine treatment directs cancer cell death through autophagy. Yet, it should be noted that Bid and Bim, which were noted to be upregulated, are known to upregulate Bax expression [50], which may explain the observed rt-qPCR results. Since BECN1 gene upregulation was also observed at a protein level, this may suggest that increased expression of BECN1 is a significant molecular effect of α-solanine treatment.

Notwithstanding, the interaction between apoptotic and autophagic proteins is dynamic and very complex. A PPI network was constructed to visualise the interactions between the proteins noted to be upregulated through proteome profiling. The interactions between BENC1/Bcl-2; Bcl-2/Bim; Bcl-2/Bid and Fas/Bid emerged and promising targets dysregulated by α-solanine. Fas ligand and Fas receptor upregulation results downstream Bid activation which subsequently acts to inhibit Bcl-2 and promote mitochondrial membrane permeabilization. Bcl-2 inhibition promotes BECN1 activation resulting in autophagy, consolidated by upregulating ATG4 and LC3A. Furthermore, thus study has identified that α-solanine induces BNIP3L upregulation which mediates mitophagy to ensure the removal of dysfunctional mitochondria and reduce oxidative damage (Figure 4.). α-solanine has the potential to modulate cell death pathways, resulting in a complex cellular response involving apoptosis, autophagy as well as mitophagy.

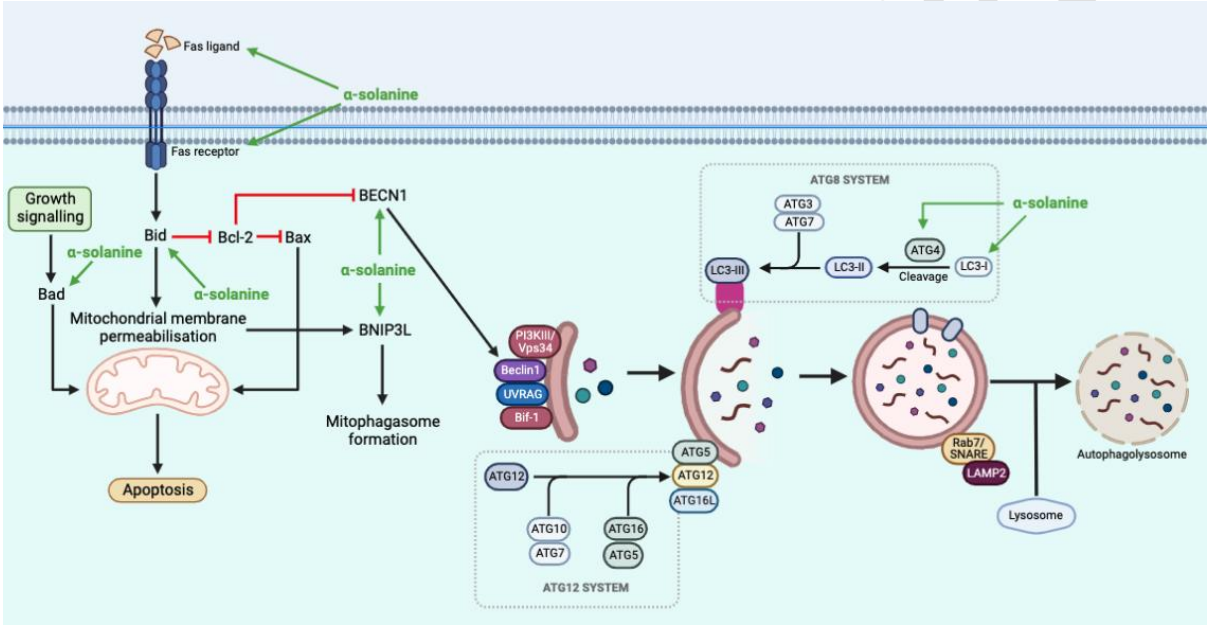


Figure 4: Proposed mode of action of α-solanine on the apoptosis-autophagy axis**.**

Limitations, and areas for improvement

The viability assays conducted in this study were carried out for 72 hours, while the published literature assessing the viability of different cancers following α-solanine treatment used 24-hour and 48-hour time durations. This difference in time duration limits the direct comparison of the results through meta-analysis and interpretation of the efficacy of α-solanine on GBM. Furthermore, The study did not achieve the IC_{50} of TMZ treatment against all three GBM cell lines. This constraint prevented inferential statistical comparisons between α-solanine and TMZ treatments, limiting the evaluation of their relative efficacy. The study did not establish a clear distinction between the effects of α-solanine and non-specific or inherent cellular responses. However, results obtained are on par with and consolidate findings of published literature. Moreover, our study was unable to assess the efficacy of α-solanine in-vivo and ex-vivo using patient derived tissue, due to constraints beyond the author's control. Notwithstanding this study serves as a reliable proof of concept on the efficacy of α-solanine against GBM.

Conclusion

This study provides insights into the potential therapeutic use of α-solanine against GBM and its impact on cell death. The study compared α-solanine with the chemotherapy drug TMZ and evaluated its cytotoxic effects and effects on GBM cell migration. α-Solanine showed cytotoxicity against both TMZ-sensitive and TMZ-resistant GBM cell lines, with similar potency to TMZ. It also demonstrated potential in reducing cell migration. The study explored the apoptosis-autophagy axis and found evidence of dysregulation of the apoptosis-autophagy axis. However, further investigation is needed to understand the specific mechanisms of cell death induced by α-solanine and its impact on invasion. The study suggests that α-solanine has promising potential as a GBM treatment, but more research is required, including *in vivo* experiments and testing on patient-derived GBM tissue, to validate its clinical applicability.

Statement and declarations

Data availability

Data will be made available upon request.

Ethical approval

Ethical approval was obtained by the Faculty research and ethics committee (FREC) of the Faculty of Medicine and Surgery at the University of Malta [MED-2022-00115].

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