Valorisation of Daffodil Waste Stream and Stabilisation of Haemanthamine by Nanoparticle Encapsulation

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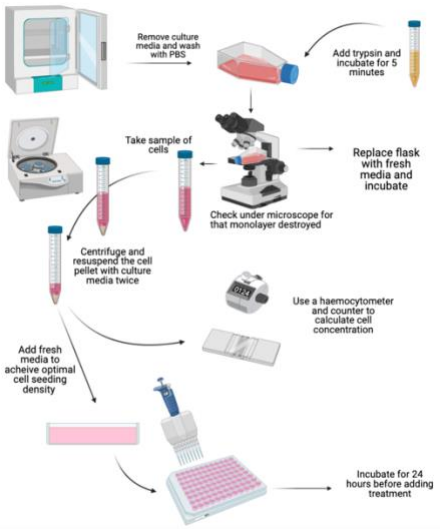
Introduction

With the prevalence of oncological diseases in the developed world rising, the discovery of new chemotherapy drugs is essential. Plants represent an important source of pharmaceutically active products. Presented here, the potential of the daffodil derived drug haemanthamine (HAE) is assessed.

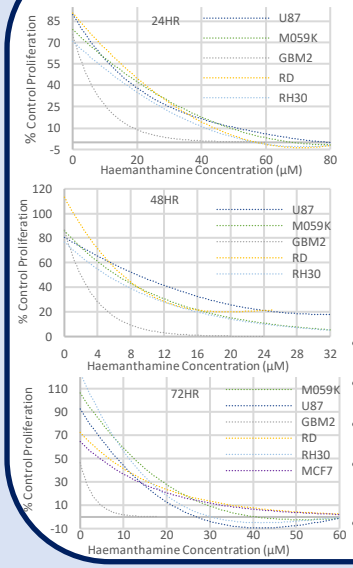
Aims and objectives

-To assess the cytotoxic effect of HAE on various cancer cell lines *in vitro* using glioblastomas (U87, M059K, GBM2), rhabdomyosarcomas (RH30, RD) and breast adenocarcinoma (MCF7)
-To improve the characteristics of HAE through nanoparticle encapsulation
-Assess the cytotoxic effect of HAE encapsulated nanoparticles on cancer cells

Cell Culture and Seeding Protocol



Cytotoxicity Test Results



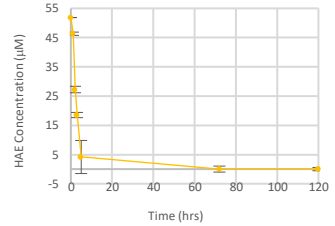
The cytotoxic effect of HAE on 6 cancer cell lines (U87, M059K, GBM2, RH30, RD and MCF7) was assessed after culturing and seeding according to the schematic protocol shown.
Cell death was evaluated using a crystal violet assay at 595 nm and quantified as % control proliferation of cells.

Key Findings:

- Each cell line experienced an exponential decay trend as HAE concentration increased
- All cell lines had half maximal inhibitory concentrations in the 1.5 – 13.9 μM range
- HAE had a less pronounced effect on normal human fibroblasts than on cancer cells
- HAE caused greatst cell death in GBM2- a paediatric brain tumour which has failed to respond to conventional treatments
- Longer treatment times produced less cell death

Haemanthamine Stability

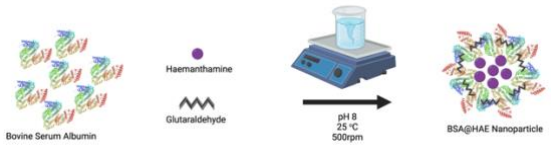
The stability of HAE was assessed by incubating the drug in Phosphate-Buffered Saline at 37°C.
UV spectrophotometry at 290 nm was used to quantify the concentration of HAE after different time intervals.
The results are displayed as HAE concentration against incubation time.



- HAE showed low stability with rapid degradation in the first 5 hours
- Drug half-life = 2.57 hours
- This would make HAE an ineffective chemotherapy drug
- Validates why longer treatment times produced reduced cell death in the cytotoxicity test
- HAE requires stablisation if it is to be an effective *in vivo* drug

Nanoparticle Encapsulation by Desolvation

Nanoparticle delivery systems can provide enhanced biocompatibility, bioavailability, active targeting and an improved stability. Bovine serum albumin (BSA) was selected as the nanocarrier due to its desirable characteristics and abundance. A schematic of the desolvation method used is shown below.

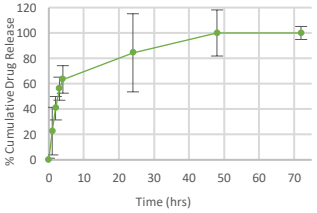


- HAE encapsulated BSA nanoparticles are denoted by BSA@HAE
- Nanoparticles were characterised in terms of size and zeta potential using Dynamic and Electrophoretic Light Scattering
- The size and zeta potential achieved represent a high colloidal stability and suggest they will have a high affinity for cell membranes and support adsorption into cells

Nanoparticle Characterisation	
Yield	57 %
Encapsulation Efficiency	93 %
Loading Capacity	5.47 μmol HAE/mg BSA
Size	272 nm
Zeta Potential	-30.1 mV

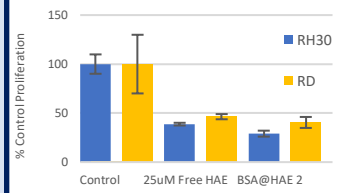
Drug Release Profile

- Drug release was investigated by incubating nanoparticles in Phosphate-Buffered Saline at 37°C
- Cumulative percentage drug release was plotted by quantifying HAE released using UV spectrophotometry
- Initial burst phase observed
- Drug release plateaued with 100% release after 48 hours
- Overall, improved drug release characteristics were achieved



Nanoparticle Cytotoxicity Evaluation

The cytotoxic effect of the produced nanoparticles was then tested on rhabdomyosarcoma cell lines.



- Drug free nanoparticles caused an increase in cell proliferation relative to control cells
- BSA@HAE nanoparticles showed a significant decrease in cell proliferation from control cells
- BSA@HAE nanoparticles produced greater cell death than free HAE of the same concentration

Conclusions

- HAE displayed effective *in vitro* anticancer activity on the 6 cancer cell lines investigated
- HAE showed greater cytotoxic on GBM2 cells
- HAE exhibited low stability with a half-life of 2.57 hours
- Nanoparticle encapsulated HAE achieved a high loading capacity and yield
- Nanoparticle size (272 ± 63 nm) and zeta potential (-30.1 ± 1 mV) represent high colloidal stability and support adsorption into cells
- Drug free nanoparticles were absorbed and lead to increased cell proliferation
- HAE encapsulated nanoparticles produced greater cell death with improved drug release characteristics
- Collectively, HAE showed great potential as a chemotherapy drug

Collaboration with Agroceutical Products

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