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GROWTH INHIBITORY AND CYTOTOXIC EFFECTS OF MIMOSINE ON HUMAN NEURONAL CELL LINES – IMR-32, U373-MG, SK-N-SH BY SRB ASSAY

Kannan R.*[1] and Sibi P. Ittiyavirah[2]

[1]Department of Pharmaceutical Sciences, Centre for Professional and Advanced Studies, Kottayam, 686631, Kerala, India.

[2]Department of Pharmaceutical Sciences, RIMSR, Centre for Professional and Advanced Studies, Kottayam, 686009, Kerala, India.

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***Corresponding Author**
Kannan R.
Department of Pharmaceutical Sciences, Centre for Professional and Advanced Studies, Kottayam, 686631, Kerala, India.

ABSTRACT

Mimosine, a non-protein amino acid, isolated from *Mimosa pudica* was evaluated for its neuroprotective effects using human cell lines IMR-32, U373-MG and SK-N-SH. The cytotoxic potential was assessed by Sulforhodamine B assay and the parameters like GI_{50}, TGI and LC_{50} were measured. The GI_{50} was found to be 55.2 and 37.3µg/ml for IMR-32 and U373-MG while that for standard Adriamycin it was less than 10µg/ml and no significant inhibition was noted on SK-N-SH cell lines. It was concluded that Mimosine exhibited a significant cytotoxic activity with fatality as evidenced by the LC_{50} values.

KEYWORDS: Mimosine, SRB assay, IMR-32, U373-MG, SK-N-SH.

INTRODUCTION

Mimosine also called Leucenol is a non-protein amino acid which is chemically similar to tyrosine. It was first isolated from the "Sensitive plant - *Mimosa pudica*" belonging to the family Fabaceae. It is also being isolated from few other species of Mimosa and in all plants belonging to the genus Leucaena.[1] The studies on Mimosa showed many potential activities including anti-inflammation, anti-viral, anti-fibrosis, anti-microbial and anti-cancer activity.[2] Restivo A et al reported the anti-proliferative effect of Mimosine on ovarian cancer cell lines and found that it was producing a significant effect.[3] Alpan R and Pardee A reported that Mimosine acts by blocking the late G-1 phase of the cell cycle on human breast cancer cell

lines.[4] Our earlier studies on adaptogenic and nootropic potential of *Mimosa pudica* using rodents prompted us to explore further on its activity.[5,6] In this study, for the first time, we reported the cytotoxic potential of Mimosine on three different human neuronal cell lines.

Sulforhodamine B assay provides a rapid and sensitive method for measuring the drug-induced effects in both attached and suspension cultures. Sulforhodamine B is a bright pink aminoxanthene dye with two sulfonic groups, which bind to protein basic amino acid residues under mildly acidic conditions and the colour development in the assay is rapid, stable and visible. The optical density of sulforhodamine B assay can be measured over a broad range of visible wavelengths in either a spectrophotometer or a 96-well plate reader. The major advantage of this assay over others is that this dye would not stain cell debris. Therefore, the sensitivity of sulforhodamine B assay is not affected by the presence of cell debris. So, it has been widely used for the cell growth studies.[7,8]

Three different cell lines IMR-32; U373-MG and SK-N-SH were used here in this study to evaluate the efficacy of Mimosine. The IMR-32 are a continuous hyperdiploid human neuroblastoma cell line and it is proved to be an excellent source for the isolation of human neuronal nAChR subunit cDNAs.[9] Acetylcholine plays a vital role in both memory and behaviour. Furthermore, it is also stated that wild-type human neuroblastoma IMR-32 cells have the ability to secrete long amyloid -protein.[10] Amyloid beta acts as a neurotoxin when gets accumulated in the brain. U373-MG a human astrocytoma cell line is proved to provoke inflammatory markers like IL-6 when triggered with certain inducers, and it is as well found that serotonin receptors can be expressed/unexpressed to modify the release of IL-6.[11] SK-N-SH is a continuously cultured human neuroblastoma cell line, which is well known for studies related to neurodegenerative diseases. It is also proved that these cell lines are capable of synthesizing multiple neurotransmitters which includes Dopamine, Nor-epinephrine, Acetylcholine, GABA, etc.[12]

MATERIALS AND METHODS

Mimosine (M0253) was purchased from Sigma-Aldrich, Inc., USA and the other chemicals used were purchased from HIMEDIA laboratories, Mumbai, India.

Evaluation of cytotoxic potential by Sulforhodamine B assay (SRB assay).

The cell lines were cultured on RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. The cells were inoculated into 96 well microtiter plates in 100 µL at plating densities depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of Mimosine.

Mimosine was initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrates (1mg/ml) was thawed and diluted to 100, 200, 400 and 800 g/ml with complete medium containing test articles. Aliquots of 10 µl of these different dilutions were added to the appropriate microtiter wells already containing 90 µl of the medium, resulting in the required final drug concentrations, i.e.10 g/ml, 20 g/ml, 40 g/ml, 80 g/ml.

After addition of Mimosine, plates were incubated under standard conditions for 48 hrs, and the assay was terminated by the addition of cold TCA. Cells were fixed *in-situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, the unbound dye was recovered and the residual dyes were removed by washing five times with 1% acetic acid. The plates were air dried. The bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm as reference wavelength.

The following parameters were estimated,

LC_{50} = Concentration of drug causing 50% cell kill.

GI_{50} = Concentration of drug causing 50% inhibition of cell growth.

TGI = Concentration of drug causing total inhibition of cell growth.

Percentage growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percentage Growth was expressed in the ratio of average absorbance of the test well to the mean absorbance of the control wells x 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition was calculated as [Ti/C] x 100 %

A growth curve was constructed using percentage control growth versus drug concentration using the growth curve the concentration of drug causing 50% inhibition of cell growth (GI_{50}) was calculated.[8,13]

RESULTS AND DISCUSSION

WHO stated that "Cancer is one of the leading cause of death worldwide and is the second most cause globally i.e., 1 in 6 deaths occurs due to cancer"[14] In our study we tested the effect of Mimosine on neuronal cell lines. Mimosine can be a promising anticancer agent as evidenced by its cytotoxic effects on various cell lines viz., lung cancer cell lines (H226, H358, H322)[15], breast cancer cell lines (MDA-MB-453)[16], pancreatic cancer cell lines.[17] Bexarotene was the first anticancer compound reported being effective in reversing the memory deficits in AD mouse model. This phenomenon shows the interlink between anticancer drugs and AD. Even though a few milestones were achieved, the research in this direction is still continuing. It is even clinically evidenced that cancer patients who had survived breast cancer had a lower risk of developing AD after using anticancer drugs.[18] This led to the new approach to developing a drug for treatment of AD.

In the present study, the growth curve of all the three different cell lines was constructed using drug concentration versus percentage control growth (Figure 1, 2 and 3). From the growth curve the parameters like LC_{50}, TGI and GI_{50} were calculated. The LC_{50} of Mimosine on all the three cell line was found to be greater than 80 where the Adriamycin produced values less than 10 (Table 1). The drug is considered fatal if it causes the death of more than 50% of cells i.e. LC_{50} values more than 50% at any concentration level.[19] Similarly, the GI_{50} was also calculated from the growth curves. Mimosine exhibited the GI_{50} value of 55.2 and 37.3 on IMR-32 and U373-MG cell lines which shows activity whereas it didn't produce activity on SK-N-SH cell lines when compared to Adriamycin (Table 1). If the compound is pure, the GI_{50} value of $\leq 10^{-6}$ (i.e. 1 µmole) or $\leq$ 10µg/ml is considered indicative of significant activity.[19] Further detailed research is much needed to elucidate the precise mechanisms of Mimosine.

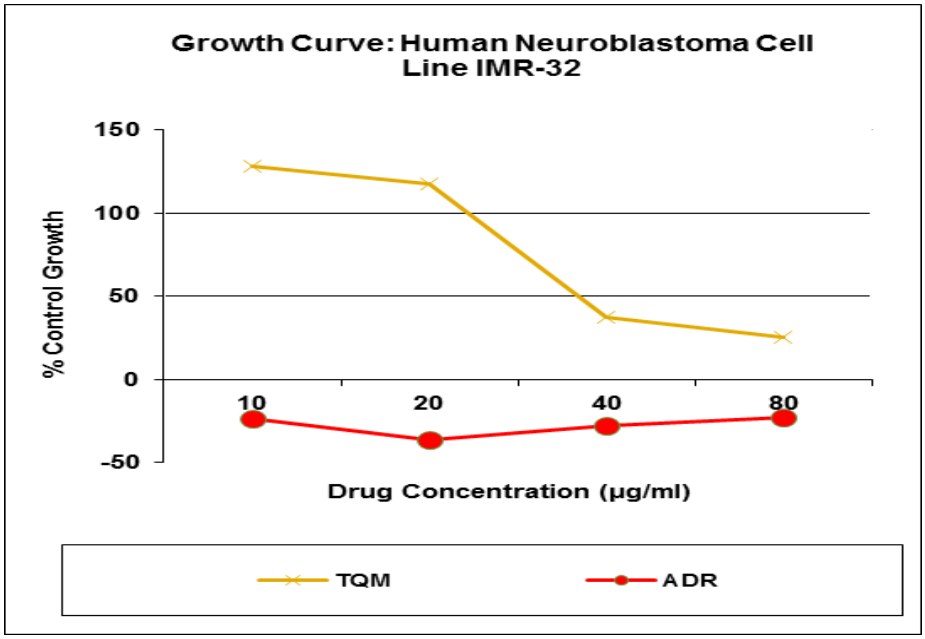

Fig 1: Growth curve of Mimosine: Human neuroblastoma cell line IMR-32 using SRB assay. TQM, ADR denotes Mimosine and Adriamycin respectively.

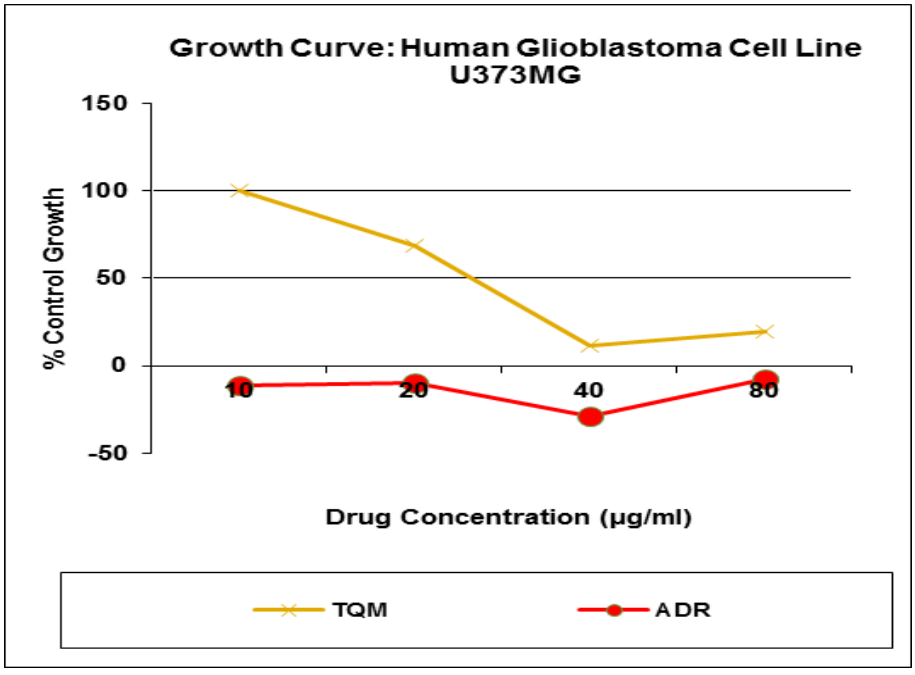

Fig 2: Growth curve of Mimosine: Human glioblastoma cell line U373MG using SRB assay. TQM, ADR denotes Mimosine and Adriamycin respectively.

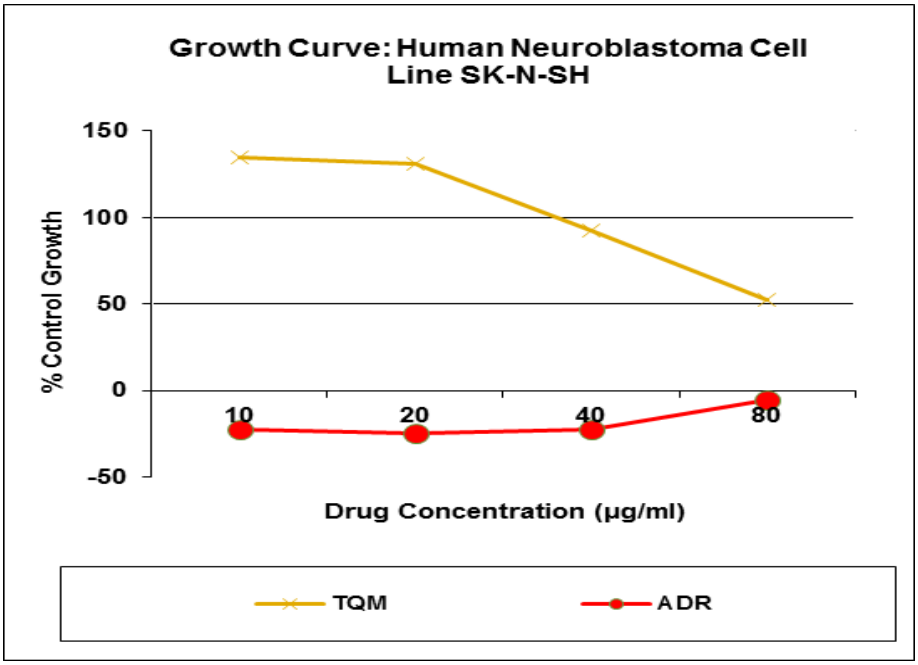

Fig 3: Growth curve of Mimosine: Human neuroblastoma cell line SK-N-SH using SRB assay. TQM, ADR denotes Mimosine and Adriamycin respectively.

Table 1: LC_{50}, TGI and GI_{50} values of Mimosine and Adriamycin.

Samples	Cell lines	LC_{50}	TGI	GI_{50}
Mimosine	IMR-32	>80	>80	55.2
	U373-MG	>80	>80	37.3
	SK-S-NH	>80	>80	>80
Adriamycin	IMR-32	<10	<10	<10
	U373-MG	<10	<10	<10
	SK-S-NH	<10	<10	<10

CONCLUSION

Our study shows that Mimosine produced cytotoxic and growth inhibitory activity on human neuroblastoma (IMR-32) and glioblastoma (U373-MG) cell lines. It showed the least cytotoxicity on SK- S-NH cell line. However, Mimosine had shown LC50 values greater than 80 for all the three cell lines which demonstrate its lethality. Hence further studies are needed to optimize the dosage formulation which could yield an effective drug for the treatment of cancer and even for the AD.

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Conflict of interest

The authors declare that there is no conflict of interest.

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