REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1	Name and designation of ICMR- IF	Dr Challa Suresh, Scientist 'E'
2	Address	Department of Biochemistry National Institute of Nutrition Indian Council of Medical Research Tarnaka, Jamai-osmania (PO) Hyderabd-500007
3	Frontline area of research in whichtraining/research was carried out	Bio-Medical Research and Toxicology
4	Name & address of Professor and host institute	Chellu S Chetty, Ph.D. Associate Vice President for Research & Sponsored Programs, Regents Distinguished Professor of Biology PO Box: 40289,Room 205, Colston Administrative Bldg Savannah State University, Savannah, GA 31404, USA E mail: chettyc@savannahstate.edu
5	Duration of fellowship	Six Months (1st jan 2015-30th June 2015)
6	**Highlights of work conducted**	
	i. Techniques/expertise acquired	a. Learnt the operation, trouble shooting and analysis of various experiments being conducted using Flow Cytometry;b. Handled several Human carcinoma cell lines viz. Melanoma, Breast, Neuroblastoma, Leukemia and epithelial cells; c. Got expertise in the designing different modules related to the testing of various synthetic Betulinic acid like derivatives, testing their toxicological competenceagainst cancer cells and also studies related to apoptosis and cytotoxicity assays. d. Designing experiments related to the find the effects of Lead and amyloid peptides on the Human brain cells at various levels of cell signaling, mitochondrial membranes and also cell cycle analysis.
	ii.Research results, including any papersprepared/submitted for publication	**ANNEXURE - I**
	iii. Proposed utilization of the experience in India	The work done in the area of Lead toxicity using FACS is very much in the priority areas of our organization, ICMR. The obtained expertise will be utilized in the effective execution of the ongoing projects and also the efforts are being made to develop Indo-US collaborative project in the area of Betulinic acid and developing different derivatives as effective therapeutic agentsto combat the different types of carcinomas.

ANNEXURE –I

A. Research Results:

1. Developing Betulinic acid like derivatives to fight against different cancer cells

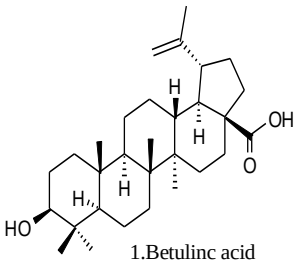
Betulinic acid (BA) is a natural product possessing abundant and favorable biological activity, including anti-cancer, anti-malarial, anti-inflammatory and anti-HIV properties, while causing minimal toxicity to unaffected cells. The full biological potency of BA cannot be fully unlocked, however, for a number of reasons, a primary one being its limited solubility in aqueous and biologically pertinent organic media.

Objectives

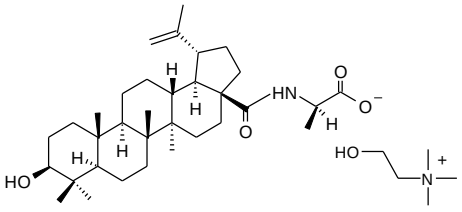
To Develop Betulinic acid like derivatives to fight against against different cancer cells

Results achieved

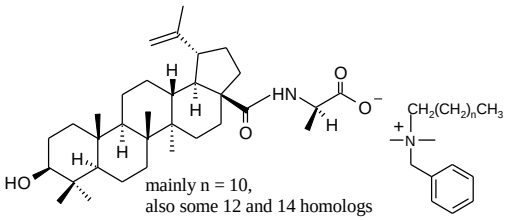
Aiming to improve the water solubility of BA without disrupting its structurally related bioactivity, we have prepared different ionic derivatives of BA. Inhibition bioassays on various carcinoma cells indicated a significant improved performance resulting from converting the BA to organic salt form. Indeed, for one particular cholinium-based derivative, its water solubility is improved more than 100 times and the half maximal inhibitory concentration (IC50) value. These encouraging results advise that additional studies of ionic BA derivatives as a therapeutic solution against various cancer cell lines. In fact, BA because of its poor water solubility prevents the effective bio-availability in the cells results in the reduced toxicity effects on the cells. To combat this particular property, we have prepared new ionic derivatives of BA with high water solubility, without losing the structural integrity and functionality of this compound. As a result, these new ionic derivatives have exhibited much higher inhibitory effects against different cancer cell lines such as Melanoma SK-MEL-2, Melanoma RPMI-7951, Leukemia K-562, melanoma A375, neuroblastoma SH-SY5Y and breast adenocarcinoma MCF7. For cell lines, the derivative 5 has a low IC_{50} value of in the range of 15-55 µM (*vs*180-220 µM respectively for betulinic acid). The high cytotoxicity of these new derivatives is directly linked to their greatly improved water solubility. Our assay method used little DMSO in aiding the dissolution of these derivatives to demonstrate the advantage of improved water solubility and to mimic the *in vivo* assay conditions. The cell viability studies based on both MTT and LDH assay methods and also apoptotic marker Caspase-3 have also confirmed the high inhibitory effect of our ionic derivatives of betulinic acid (particularly 4 and 5) against different cancer cells.



1.Betulinc acid

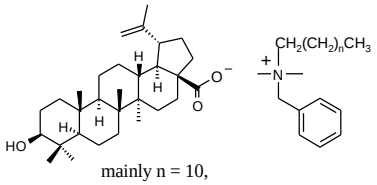


cholinium salt of betulinic acid-glycine [cholinium][BA-Gly] (**2**)



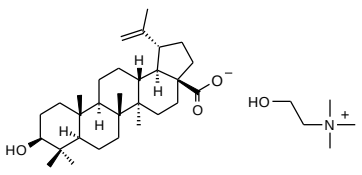
mainly n = 10,
also some 12 and 14 homologs

benzalkonium salt of betulinic acid-glycine [benzalkonium][BA-Gly] (**3**)



mainly n = 10,
also some 12 and 14 homologs

[benzalkonium][betulinate] (**4**)



[cholinium][betulinate] (**5**)

2. Effects of combination of amyloid peptides (Aβ 1-40 and 25-35) and lead (Pb) on SH-SY5Y neuroblastoma cells (part of the ongoing project work performed using FACS)

Aggregated Amyloid peptides, a major component of senile plaques has been considered to play a very important and crucial role in the development and neuro-pathogenesis of Alzheimer's disease (AD). In addition, environmental toxins are among the risk factors that may contribute to the development of AD. Among the toxins Pb is considered to be a widely used heavy metal in day-to-day life. Studies related to the synergistic effects of Pb in combination with amyloid peptides are very scanty and needs to be investigated. Even though, Aβ peptides and Pb are individually found to be toxic to brain cells, the effect of combination of these two could be vulnerable. Hence, the present study has the

Objectives

To find the effects of Pb in the AD simulated conditions in terms of ROS generation, cell cycle analysis and apoptosis *in vitro* conditions in the Human neuroblastoma SH-SY5Y cells.

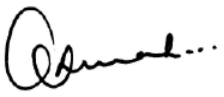
Results achieved

In the present study, the amyloid peptides Aβ1-40 (20μM to 120μM) and Aβ25-35 (2.5μM to 15μM) were exposed to the SH-SY5Y neuroblastoma cells for 24 and 48 hrs both individually and in combination. The cell viability of cells were significantly reduced and 50% decrease in cell viability (IC50) was observed at 60 μM Aβ1-40 and 7.5 μM for Aβ (25-35). Cells were treated for 48 hrs with lead alone (0.01 to 10 μM) showed a significant difference in the decrease of the viability and IC50 was observed to be at 5 μM. To find the combination effects, the cells were pretreated with Aβ(1-40:60μM,25-35:7.5μM) and Lead (Pb) (5μM) and the viability was significantly altered and combination of Aβ with Pb was observed to be effective. Other parameters like ROS generation, Induction of Apoptosis and the cell cycle analysis iwere performed using FACS. The results indicates that, the Pb alone is very effective in generating the cytotoxicity when compared with the Amyloid peptides individually and combinations. But, the Pb and AP combo was to aggravate the death of cells. Similar kind of situation was observed in the early induction of apoptosis. The eary apoptotic was 13.9% in Pb alone, 12.8 % in the AP(1-40), 0.7% in AP (25-35), Pb+AP(1-40) is 22.2% PB+AP (25-35) is 9.8%, PB +(1-40)+(25-35) was found to be 24.9 %. Similarly, the cell cycle cycle ananlysis indicated the combination of Pb+(1-40)+(25-35) has showed 62.8 %, 27.09 % and10.08 % in the G1,G2 and S phase.Findings suggests that toxic effects of amyloid peptides (1-40 and 25-35) in combination with Pb were visible and was significant in aggravating the clinical pathological manifestation reflecting in oxidative stress, neuronal apoptosis and in altering the cell cycle events.

B. Papersprepared/submitted for publication:

i. Synthesis and exploring the possible anti-cancer effects of Betulinic acid like derivatives. Communicated to "Food and Chemical Toxicology"

ii. Deleterious effects of the Pb-Amyloid combo in altering the cell cycle and its role in inducing apoptosis in the Human Brain cells. Communicated to "Cellular and Molecular Biology Letters".



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