PROJECT REQUIREMENT:

- To determine the cell proliferation study on HepG2 and L929cell lines, 1sample was received.
- The sample details as below:

Sl. No.	Sample Name/Code	Concentrations	Cell lines
1	GAIN-IT	5 (20,40,60,80 and 100uG/ml)	HepG2 and L929
Additional details of compound:		• Color: Pale brownish • Powder form • Batch No: GT/101 • Mfg date: 03/2022 • Expiry date: 02/2025	

Table 1: Details of Sample received for the study

BACKGROUND OF THE STUDY:

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye, MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley et al and Mosamann et al)

MATERIALS:

1. Cell lines:

   a) HepG2-Human Hepatomacell lines (NCCS, Pune)

   b) L929-Mouse embryo fibroblast cell line (NCCS, Pune)

2. Cell culture medium: DMEM- high Glucose - (#AL111, Himedia)
3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
4. Fetal Bovine Serum (#RM10432, Himedia)
5. MTT Reagent (5 mg/ml) (# 4060 Himedia)
6. DMSO (#PHR1309, Sigma)
7. D-PBS (#TL1006, Himedia)
8. 96-well plate for culturing the cells (From Corning,USA)
9. T25 flask (# 12556009, Biolite - Thermo)

10. 50 ml centrifuge tubes (# 546043 TARSON)

11. 1.5 ml centrifuge tubes (TARSON)

12. 10 ml serological pipettes (TARSON)

13. 10 to 1000ul tips (TARSON)

EQUIPMENTS:

1. Centrifuge (Remi: R-8^{o}C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted binocular biological microscope (Biolinkz, India)
4. Biosafety hood (Biobase, China)
5. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)
6. 96well plate reader (ELX-800, BioTek, CA, USA)

ASSAY CONTROLS:

(i) Medium control (medium without cells)
(ii) Negative control (medium with cells but without the experimental drug/compound)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

MAINTENANCE OF CELL LINES:

The HepG2 (Human hepatoma cell line) and L929 (Mouse embryo fibroblast cell line) were purchased from NCCS, Pune, India. The cells were maintained in DMEM high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days.

STEPS FOLLOWED:

1. Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours.
2. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet).
3. Incubate the plate for 24hrs at 37°C in a 5% CO_2 incubator.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminium foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
7. Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO).
8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.

9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength.
10. % Cell viability is calculated using below formula:

% cell viability= [Mean abs of treated cells/Mean abs of Untreated cells] x 100

RESULTS:

In this study, given 1 test compound was evaluated to analyse the cell proliferation effect on HepG2 and L929 cells. The concentrations of the test compound used to treat the cells are as follows:

Sl.No	Culture condition	Cell lines	Concentration treated to cells
1	Untreated	HepG2 and L929	No treatment
2	Blank	-	Only Media without cells
3	Std control	HepG2 and L929	1 (1ug/ml)
4	Test compounds	HepG2 and L929	5 (20,40,60,80 and 100ug/ml)

Table 2: Details of drug treatment to respective cell lines used for the study

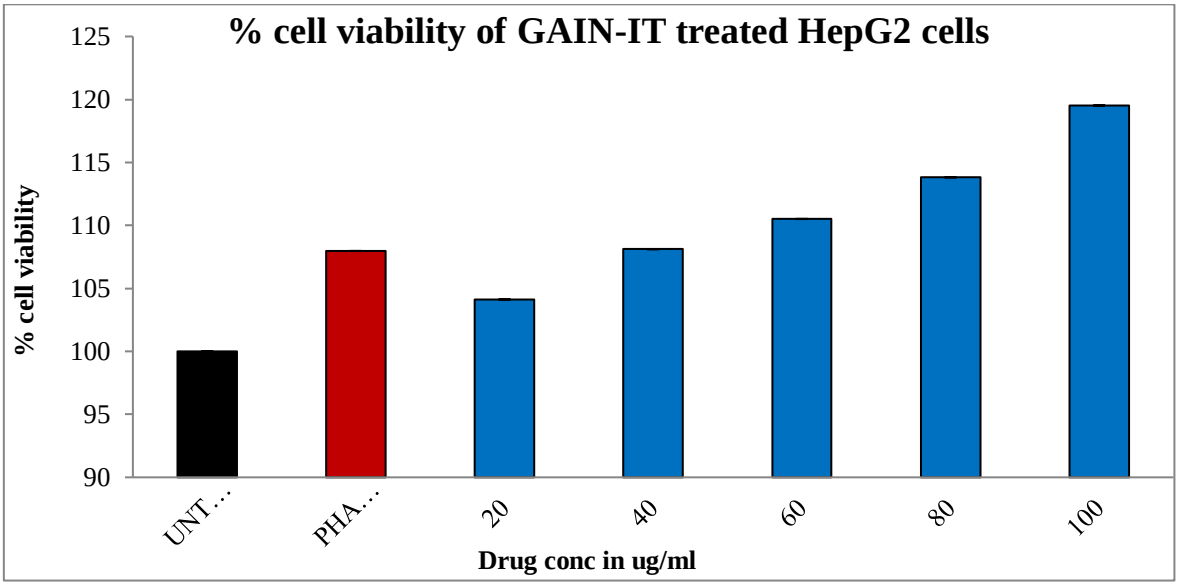
OBSERVATIONS& CONCLUSIONS:

Culture condition	HepG2	L929
Untreated	100	100
PHA 1ug/ml	108	107.32
20ug/ml	104	102.06
40ug/ml	108	105.59
60ug/ml	110.5	108.78
80ug/ml	113.8	115.60
100ug/ml	119.5	121.86

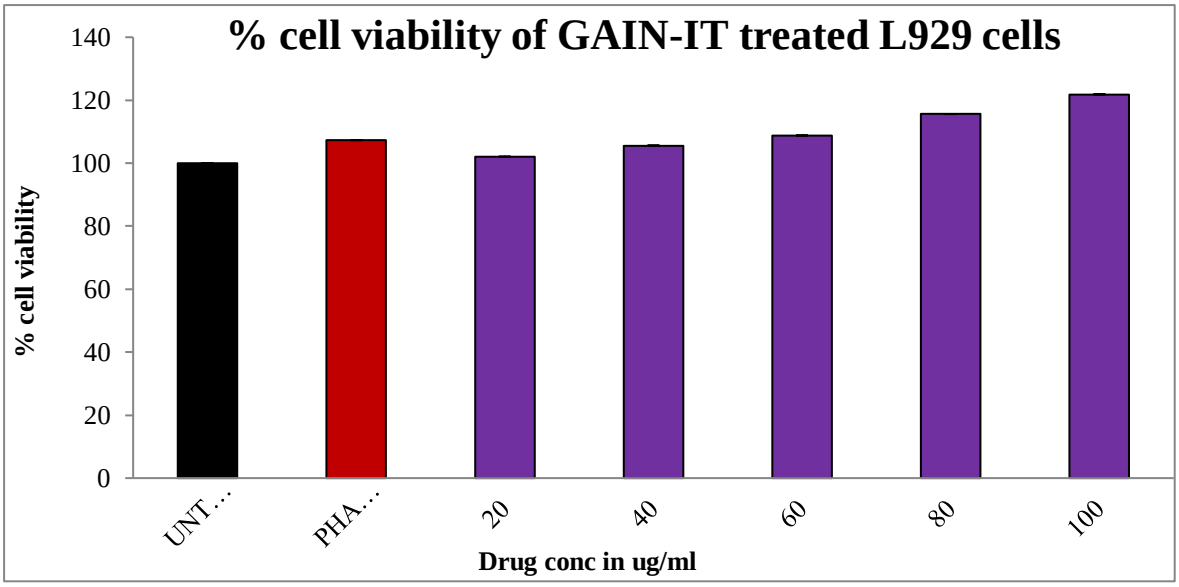
Table-3: % cell viability values of GAIN-IT against HepG2 and L929 cells after the treatment period of 24hrs.

- The results of cytotoxicity study performed by MTT assay suggest that the given test compound, GAIN-IT was effective cell proliferative in nature on Human liver (HepG2) cells as well as Mouse embryo fibroblast (L929) which showed cell growth on dose dependent manner.
- The absorbance readings with calculations are enclosed in MS excel format.
- The direct microscopic observations of drug treated images of test compound after 24 hours of incubation were enclosed in the separate folder along with this report.

OVERLAY GRAPH:



Graph 1: % cell viability values of GAIN-IT on HepG2 cells after the incubation period of 24hrs.



Graph 2: % cell viability values of GAIN-IT on L929 cells after the incubation period of 24hrs.

CONCLUSION:

The MTT assay results suggest us that the given test compound, was non-toxic as well as cell proliferative in nature on human liver cells and Mouse embryo fibroblast cells by taking PHA as a standard control for the study. Vital growth factors like VEGF, EGF, Collagen and Elastase play a vital role in process of Muscle gain, wound healing properties etc., to find the molecular mechanism of action of given test compound (GAIN-IT) behind its cell proliferative action further in vitro level studies has to be performed.

REFERENCES:

1. MTT Cell Proliferation Assay Instruction Guide – ATCC, VA, USA www.atcc.org
2. GerlierD., and N. ThoS2set. J. Immunol. Methods 94: 57-63, 1986. Alley, M.C., et al.
3. Cancer Res. 48: 589-601, 1988. Mosmann, T. J. Immunol. Methods 65: 55-63, 1983.
4. Alley, M. C., Scudiere, D. A., Monks, A., Czerwinski, M., Shoemaker, R. II., and Boyd, M. R. Validation of an automated microculturetetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. Proc. Am. Assoc. Cancer Res., 27: 389, 1986
5. *http://himedialabs.com/TD/CCK003.pdf*
6. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65: 55–63.