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Cytotoxic activity of Kaandha chendooram a Siddha medicine on HEPG2 cancer cell lines [Hepato Cellular Adenocarcinoma]

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Abstract

Siddha system of medicine is a traditional and indegionous system (knowledge) inherited from guru parambara teachings by thiru moolar lineage and nanthi devar lineage. The mystic zenith is alchemy ,which extensively involves transmuting of metal to alloy ,minerals, extraction of metals. The usage of heavy mettalic preparations and practices have a long run. Purification by specific process is the essential thing for consuming the inorganic drugs, so the no other way toxicity could happen. several anti dote procedure also included and documented, meagre the toxic can happen when it is not strictly adhered with pathiya . Theran a known sage for his tremendous contribution explain apathiya thodam. (Diet induce, drug induced toxicity) kaandham is one among the medicines serve as elixer in certain conditions Siddha emphasises kaandam (magnatetite) always better than iron. Kandam is an uparasa serves as an elixer in treating malignancy. Kaanda cheendooram cures, anaemia, anasarca hepatomegaly, cirrhosis, peritonitis jaundice, liver mallignancy, ultimately, abdominal tumour 'sthe present study is a n attempt to scientific validation of kaandda chendooram by studying *in vitro* anti cancerous effect of kaandha chendooram against to hepatoCellular adenocarcinoma, result shows kaandha chendooram pocess cyto toxicity withIC50 concentration at 212.68ug/ml.

Keywords: Kaanda chendooram, magnatite, siddha medicine, hepato cellular carcinomas, elixer, anti caner, medicine for liver disease

Introduction

Kaandha chenndoiram is a age old preparation extremely indicated for liver disease and hepatobilliary disease, anaemia (visha paandu) kavisaikatt i(liver tumours) associated with jaundice, dropsy, manthakatti (cirrhosis), peritonitis, scrotal swelling intestinal tumours and other complication involving underlying liver malignancy. kaandha chendooram serves as a key ingredients of kandakarisalai chendooram, oosikanda chendooraam, jeevaloka chendooram, kamalai chendooram, indicated for for all liver disease. Siddha doctrine satkariya vadham advocates kaandha chendooram formulation will be work for curative process of kavisaikatti (hepatocellular carcinoma).

Kaandham the literal meaning, this is a n element which sustinence life so that this medicine is renowned as a sustinator of life. Sage Bohar, agathiyar, extools the virtue of kaandha chendooram Kaandham chendooram is composed of sulphur , *Eclipta prostata*, *Calotropis procera*, *Plumbago xeylanica* as per our literature hepatocellular carcinoma is a diseases caused by iyyam humour this kaandham (magnetite)its self capable of curing devasting iyya.

siddha literatures extensively documented the usage of kaandha chendooram for all hepatobilliary disease associated with jaundice and its complication. 'shepatocellular carcinoma is primary a malignant tumour of liver it is one of the most common malignancy in adult life, studies reported that 10 %of death attributed by it. Viral hepatitis, chirrosis, alcohlism, dietary carcinogen a flotoxin, haemochromatosis. these are major influential factor for hc development

Objective

To determine Cytotoxicity Study on HepG2 Cell lines by kanda chendooram

Table 1: Details of Sample Received

Sl. No.	Sample Name/Code	Concentrations	Cell line
1	TEST	5(25,50,100,200,400uG/mL)	HepG2

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, M. C et al., 1986, Mosmann et al., 1983).

Materials and Methods

Cell lines:

a) HepG2-Human Hepatocellular Adenocarcinoma cell line (From NCCS, Pune)

b.Cell culture medium: DMEM- High Glucose -(#AL111,Himedia)
c.Adjustable multichannel pipettes and a pipettor (Benchtop,USA)
d.Fetal Bovine Serum (#RM10432,Himedia)
e.MTT Reagent (5 mg/ml) (# 4060Himedia)
f.DMSO (#PHR1309,Sigma)
g.Camptothecin (#C9911,Sigma)
h.D-PBS (#TL1006,Himedia)
I.96-well plate for culturing the cells (FromCorning,USA)
j.T25 flask (# 12556009, Biolite -Thermo)
k.50 ml centrifuge tubes (# 546043TORSON)
l.1.5 ml centrifuge tubes(TORSON)
m.10 ml serological pipettes(TORSON)
n.10 to 1000 ul tips(TORSON)

Equipments:

1. Centrifuge (Remi: R-8^{O}C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
Inverted microscope(Biolink)
37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

Assay controls:

Medium control (medium withoutcells)
Negative control (medium with cells but without the experimentaldrug/compound)
Positive control (medium with cells and 10ug of Camptothecin)

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.

Steps followed:

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 24hours.

Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet).

Incubate the plate for 24 hrs at 37°C in a 5% CO_2 atmosphere.

After the incubation period, take out the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.

Wrap the plate with aluminium foil to avoid exposure to light.

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.)

Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO).

Gentle stirringinagy ratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.

Read the absorbance on a spectrophotometer or an ELISA reader at 570 nm and 630nm used as reference wavelength.

The IC50 value was determined by using linear regression equation i.e. Y=Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

Formulae used:

% of cell Viability

$$\frac{\text{Mean OD}}{\text{(Sample 7 Blank) (Control 7 Bank)}} \text{ X } 100$$

Concentrations used for the study:

In this study, 1 Test Compound is evaluated to check the Cytotoxicity Study on the 1 cell line namely, HepG2. The used Concentrations of the compound to treat the cells as follows:

Table 2: Details of Drug Treatment with different concentrations to the respective Cell line used for the study.

Sl.No	Test Compounds	Cell Line	Concentration treated to cells
1	Untreated	HepG2	No treatment
2	Standard (Camptothecin)	HepG2	10ug
3	Blank	-	Only Media without cells
4	TEST[kaandha cheendooram]	HepG2	5(25,50,100,200,400uG/mL)

Observations

The direct Microscopic Observations of Drug Treated Images of HepG2 Cell line by Inverted Biological Microscope with the magnification of 10X after incubation of 24hours were enclosed in the separate folder with this report.

Table 3: Table showing the IC 50 Concentrations of the Test Compound namely TEST against the HepG2 Cells

Sl.No	Sample Code	IC $_{50}$ (uG/mL)
1	TEST	212.68

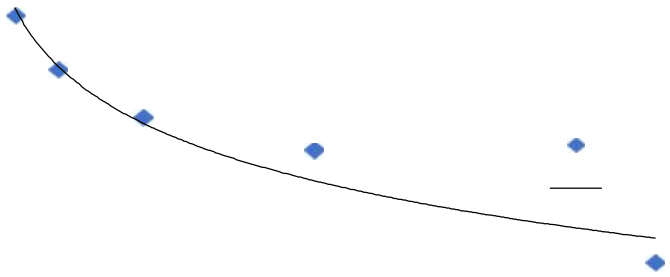
Calculations:

Test [Kaandha cheendooram] vs HepG2 Cellline

Test [Kaandha cheendooram] against the Human Liver Cancer Cell line(HepG2)

Table.4: Table showing the Absorbance Readings at 570nm in ELISA Plate Reader of the compound kaandha chendooram against the HepG2 Cell line

Concentration (uG)	Abs Reading 1	Abs Reading 2	Mean Abs	Mean Abs (Sample-Blank)	% Cell Viability
Blank	0.045	0.05	0.0475	0	0
Cell Control	0.891	0.897	0.894	0.8465	100
Std Control	0.464	0.469	0.4665	0.419	49.49793266
25	0.846	0.84	0.843	0.7955	93.97519197
50	0.722	0.728	0.725	0.6775	80.03544005
100	0.617	0.623	0.62	0.5725	67.63142351
200	0.546	0.549	0.5475	0.5	59.06674542
400	0.301	0.307	0.304	0.2565	30.3012404

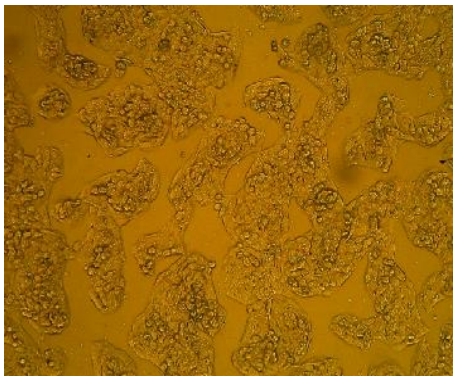


Graph 2: Scatter Graph showing the % of Cell Viability of HepG2 Cell line against the Sample TEST and the Inhibitory Concentration (IC50 Value) is 212.68µg/mL.

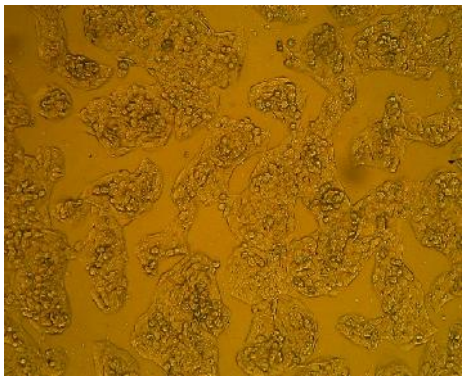
Results

The Observations in Statistical data of Cell Cytotoxicity Study by ELISA Reader suggesting us that against HepG2 cells, given Test Compound [Kaandha chendooram] TEST showing cytotoxic potential properties with the IC50 Concentration at 212.68uG/mL compared to the Standard Drug, Camptothecin with IC_{50} concentration at 10ug used for the study.

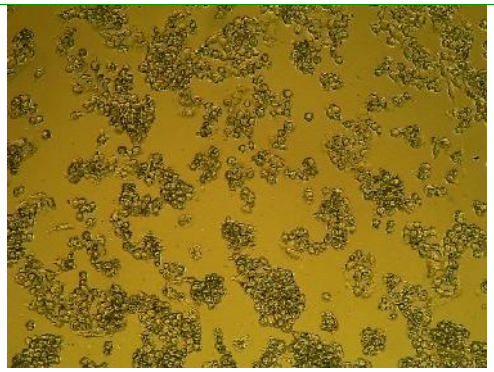
The Results suggesting us that the Test Compound [Kaandha cheendooram], TEST having Good Cytotoxic potential capacity against Human Liver Cancer Cells.



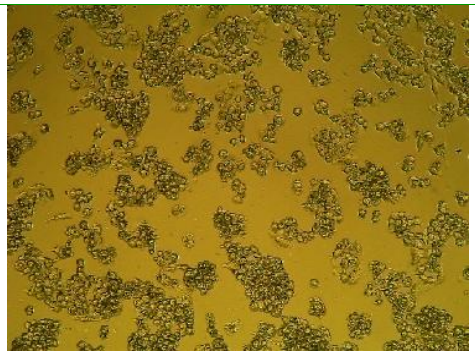
HepG2 cell control 2 image



Hepg2 cell control image 1

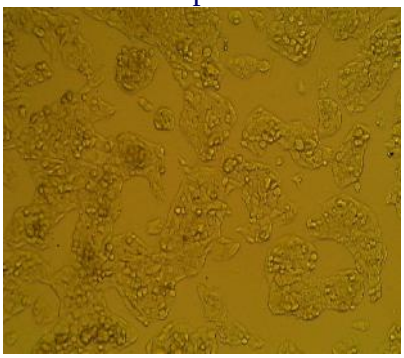


HepG2 Cell standard 1 image



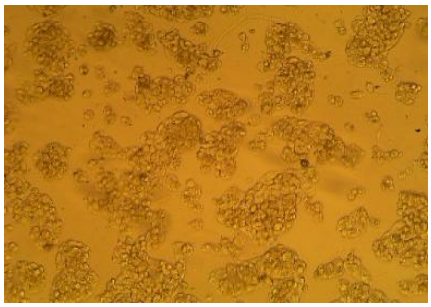
Hepg2 cell standared2 image

HepG2

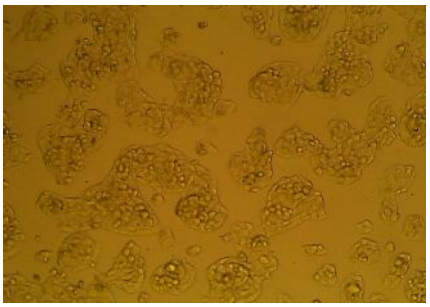


HepG2 test [Kaandha cheendooram]

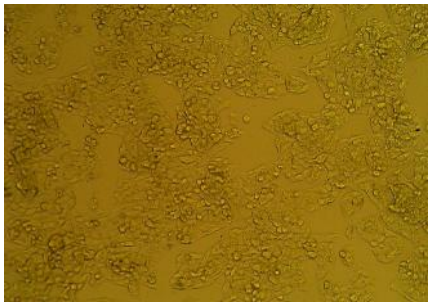
HepG2 test

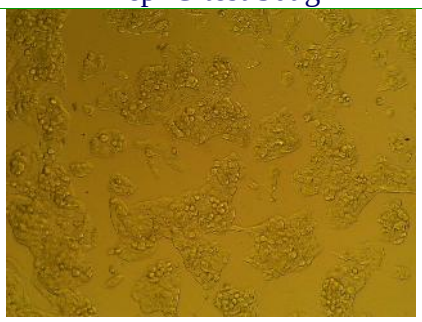


HepG2 test 25ug

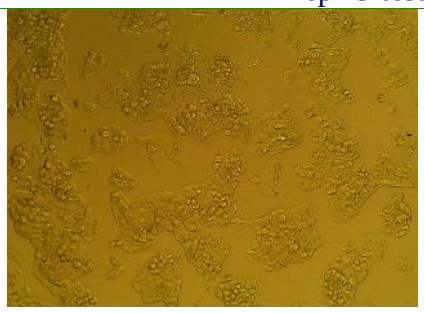


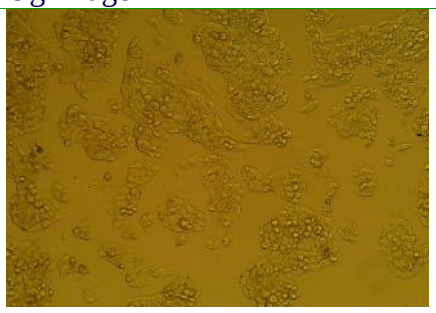
Hep2G test 50ug



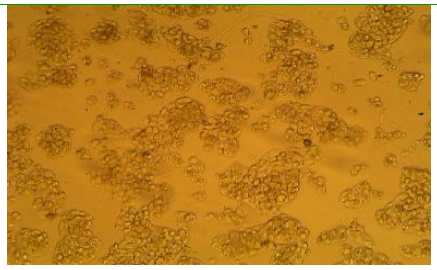


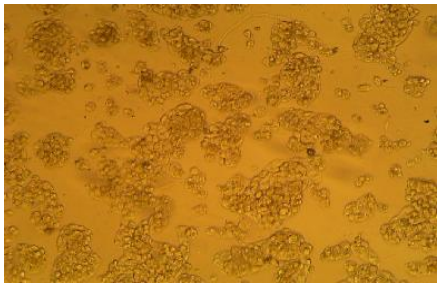
Hep2G test 100 Ug image 2

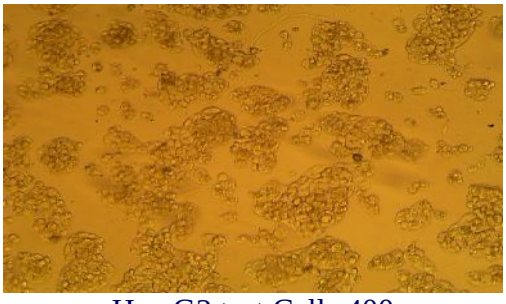




Hep2G test 200Ug image 1







Hep G2 test Cells 400

Discussion

Nowadays a modified lifestyle shows increasing in non alcoholic fatty liver disease, chirrosis and hepatocellular adenocarcinoma, results shown kaandha cendhuram is potential with IC_{50} IC_{50} the half maximal inhibitory concentration is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function . the quantitative measure indicates how much particular drug needed to inhibit a given biological process. It is commonly used as a measure of antagonistic drug potency in pharmacological research, according to FDA IC_{50}- represents the concentration of the drug that is required for inhibiting *in vitro,* it is also comparable to an EC_{50} that's is plasma concentration for a maximum effect in vivo.

Conclusion

Despite of many advances of modern medicine like surgical therapy, liver section, cryosurgery radiofrequency ablation liver transplant employed on the treatment of hepatocellular adenocarcinoma is always unsatisfactory this Kaandha chendooram a less laborious and cost effective may be available in the management of hepatocellularadeno carcinoma in near future it also prevent the disease when it is employed in earlier. siddhars ideology wins again.

Given Test compound, TEST showing IC_{50} concentration (The Concentration of the Compound have the capacity to kill 50% of Viable Cells) against the HepG2 Cells at the 212.68uG/mL respectively after the treatment of 24hours of incubation at 37°C temperature.

The observations strongly suggesting us that the Test compound namely, TEST may have possible therapeutic potential against Human Liver Cancer Cells based on the dosage of the Drug after the incubation period of 24hours.

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