Research Article

Cytotoxicity Activity of *Gymnosporia montana* on Hepatocellular Carcinoma Cell Line (HEP G_2)

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ABSTRACT

Ethanomedicinal plant like Gymnosporia montana belonging to the family Celeastraceae commonly known as Vikalo in Gujarat. Ethanomedicinally fresh leaves of Vikalo are chewed in trible regions of Gujarat to cure jaundice. The study was aimed to evaluate the cytotoxic activity of selected ethanomedicinal plants on HepG2 (Hepatocellular Carcinoma) cell line. Differnt extracts of leaf of Gymnosporia Montana were prepared. These extracts were tested for its inhibitory effect on HepG2 Cell Line at different ranges of concentration. The percentage viability of the cell line was carried out by using Trypan blue dye exclusion method. The cytotoxicity of plant extracts on HepG2 cells was evaluated by MTT assay. All the plant extracts show very less cytotoxic effect on HepG2. Hence Gymnosporia montana plant extracts can be taken for further studies as hepatoprotective activity.

Keywords: Cytotoxicity Activity, MTT Assay, ethanomedicinal plants, Hep G2 cell line.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases [1].

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis[2].

Hepatocellular carcinoma (HCC) is one of the malignancies with increasing incidence. Though there have been several curative methods for the disease, but the survival solely depends on the tumour location and the underlying liver disease, cirrhosis. There has been urgent need for the treatment of HCC to prevent its occurrence or its reoccurrence. Herbal compounds are known to play a major impact in all the stages of HCC. Therefore, there has been an increase in the research for the use of plant derived compounds as potential hepatoprotective agents against HCC for a novel drug development.Cells from the HepG2 cell line are known to retain differentiated parenchymal functions of normal hepatocytes, including the expression of P450 isoenzymes thus permit long-term studies to be performed.

The *Celestraceae* family, commonly known as the bittersweet family, consists of around 100 genera and 1300 species, mainly in tropical regions. This family contains several ones described to be useful in folk medicine. Many characteristic bioactive compounds have been reported from this family. Polyester sesquiterpene and pyridine-sesquiterpene alkaloids with insect antifeedant or insecticidal properties have been isolated from some species and recently sesquiterpene pyridine alkaloids with immunosuppressive or antitumor activities have also been described. Diterpene triepoxides with potent antileukemic and immunosuppressive activities and triterpenoid quinonemethides named as "Celastroloids" with antibiotic and cytostatic activities have been isolated from species of the *Celesteraceae* family[3].

Family *Celastraceae* contain about 90-100 genera and 1,300 species of vines, shrubs, usually of 1-9 m height with axillary or terminating short branches, glabrous, without latex. The great majority of the genera are tropical, with only Celastrus (the stuff vines), Euonymus (the spindles) and Maytenus widespread in temperate climates[4]**.** Leaves are alternate or opposite, simple and generally stipulate, flowers are bisexual and normally cymose, and fruit are 1-3 seeded, capsule or berry in *Celastraceae* family.

Gymnosporia montana (known as Vikro), occurring throughout the arid, dry areas of India, is traditionally claimed to be useful in various ailments. Very few reports

on pharmacological activity of *Gymnosporia montana* are available. On the basis of its traditional and folk-lore claim of being useful in jaundice and inflammation, researchers have evaluated its leaf extracts for possible antiinflammatory and hepatoprotective activities. Hence in present study cytotoxicity of *Gymnosporia Montana* were assess on Hep G2 cell line to prove its hepatoprotective activity[5].

MATERIALS AND METHODS

Collection and authentication of selected ethanomedicinal plant material

Leaf of *Gymnosporia montana* were collected when it fully grown.The raw material were dried under shade and reduced mechanically to moderate coarse powder.

Preparation of extracts[6]

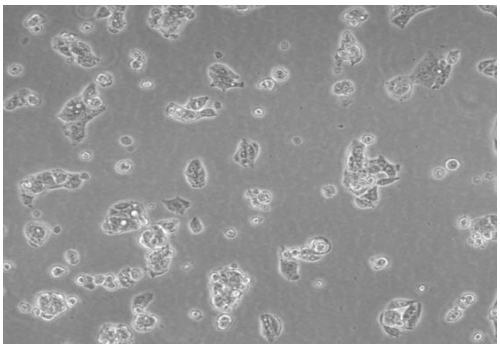
20 gm of powder of leaf of *Gymnosporia montana* were taken to prepare its different extracts. Aqueous, alcoholic and Hydro-alcoholic extracts were prepared by maceration of powder material for 48 hours. Solvents were removed by rota evaporator. Percentage yield were calculated.

Cell lines used for Cytotoxicity Screening [7]

15 years adolescent male hepatic carcinoma cell (Hep G2), Details of cell line as mentioned below:

Culture type

Organism	:	Homo sapiens, human
Tissue	:	Liver
Morphology	:	Epithelial
Culture Properties	:	Adherent
Bio safety Level	:	1



Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin (Sigma Aldrich) EDTA, Glucose and antibiotics (Hi-Media Laboratories Ltd., Mumbai) Dimethyl Sulfoxide (DMSO) and Propanol (E. Merck Ltd., Mumbai, India). Cell lines and culture medium HepG2 (human hepatocarcinoma) (National Centre for Cell Sciences (NCCS)), Pune, India.

Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solution

For Cytotoxicity studies, weighed extract was dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from the stock to carry out cytotoxic studies.

CYTOTOXICITY ASSAY

Trypan Blue Dye Exclusion Technique[8]

Trypan Blue is a blue acid dye that has two azo chromophore groups. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan Blue is an essential dye, use in estimating the number of viable cells present in a population.

Make a cell suspension in a fixed volume of cells (e.g. 1ml). Although an aseptic technique is not essential in all stages of this procedure, it is good laboratory practice to maintain sterility throughout the procedure17. Take 50uL of cell suspension and mix it with an equal volume of trypan blue. Mix solution well using a pipette. Transfer to a hemocytometer and count the live cell as clear form and dead cell as blue cells. After staining with trypan blue solution counting should commence in less than 5minutes as after that time the cells will begin to take up the dye. Using a pipette place some of the cell suspension: trypan blue mixture into the hemocytometer and overlay with a cover slip.

The cell suspension will pass under the cover slip by capillary action unless there is an air bubble.

Make sure the wells are not overfilled and that the cover slip is not moved once it is placed on the grid and the cell solution is added. Place the hemocytometer on the stage of an inverted microscope.

Adjust focus and power until a single counting square fills the field. Calculate the number of cells per ml, and the total number of cells, using the following formula18.

Calculate percent viability by using formula:

% viability = (live cell count/total cell count)*100

MTT ASSAY[9, 10]

Microculture tetrazolium (MTT) assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3(4,

5dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically.

Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakh cells/ml using medium containing 10% fetal bovine serum.

Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 hour at 37°C and in 5% CO2. Vero cell line was treated with different plant extracts at various concentrations (1000µg/ml, 500µg/ml and 100µg/ml) for 48 hours. Isoniazid +Rifampicin were used as a Positive Standard and The DMSO treated cells served as control.

Cells were then treated with MTT reagent (0.5 mg/ml as final concentration, i.e. 20µl/well of stock) for 4 h at 37°C. All the media and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; thiazolyl blue) reagent was removed from the wells and DMSO (200 µl) was added to each well to dissolve the formazan crystals.

The optical density (OD) was recorded at 570 nm in a Microplate (ELISA) reader.21

Percentage of cell viability was determined as (Avg. OD of treated cells/Avg. OD of control cells) ×100.

% Growth inhibition = 100 - [Mean OD of individual test group/Mean OD of control group × 100].

RESULTS AND DISCUSSION

In-Vitro assay of selected ethanomedicinal plants were carried out for their confirmation of hepatoprotective effect on Hep G_2 cell line. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique.

The cytotoxicity activity is carried out by using MTT assay. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.

Table 1: Percentage cell viability and characterization of cell line

Cell line	% viability	Live Cell count	Total cell count
Hep G_2	89.65%	$1.82*10^5$	$2.03*10^5$

Percentage cell viability of cell line was carried out by using trypan blue dye exclusion technique. From the Table 1, it shows that the % viability of Hep G_2 cell line is 89.65%, which is most suitable to perform cytoxicity study.

The cytotoxicity study was carried out for plant extracts. These extracts were screened for its cytotoxicity against Hep G_2 cell line at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay.

Results of % cytotoxicity of Hep G_2 cell line of all three extracts of leaf of *Gymnosporia montana* are tabulated in Table 2. The percentage viability was found to be increasing with increasing concentration of test compounds.

Table 2: % Viability of Hep G_2 cell line of leaf extracts of *Gymnosporia montana*

Different extracts of leaf of *Gymnosporia montana*	Concentration		
	100 µl	500 µl	1000 µl
Aqueous extract	56.23%	60.89%	67.17%
Hydro-alcoholic Extract	62.38%	71.44%	79.62%
Alcoholic Extract	47.3%	51.73%	55.36%

The above study shows that selected plants do not have any significant cytotoxicity on the Hep G_2 cell line. Hence the hydro-alcoholic extracts of leaf of *Gymnosporia Montana* is hepatoprotective. Further studies using more specific methods are required to explore the constituents responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and prevention of liver diseases.

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