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Cytotoxicity study of Medicinal Plant – Triumfetta Rhomboidea

Siddhi Mhatre[1], Willy Shah[2], Suhas Janwadkar[3], Siddhesh Mangaonkar[4]
1 S.D. Arts, V.S. Apte Commerce and M.H. Mehta Science college, Palghar, Dist.- Palghar
Email: psiddhi733@gmail.com Mobile No :9370675530

Abstract:

The present investigation was done to evaluate cytotoxicity of methanolic extract of medicinal plants, Triumfetta Rhomboidea on 3T3 mice fibroblast cell line. The plant sample was collected from different regions of Saphale, Maharashtra. Triumfetta Rhomboidea has various medicinal applications and therefore it becomes necessary to carry out the cytotoxic effects in its content. The cytotoxic activity of Triumfetta Rhomboidea on 3T3 mice fibroblast cell line was evaluated by using sulforhodamine B assays. The SRB assay has been used to investigate cytotoxicity in cell-based studies. The cell viability was calculated and the observed result showed that the plant extracts did not show any toxic effects till 1.0mg/ml.

Keywords: Triumfetta Rhomboidea, cytotoxic activity, sulforhodamine B assays

Introduction:

Triumfetta rhomboidea L. (Tiliaceae) is an annual herb; distributed in India, Ceylon, Malaya, Africa and America. Various parts of this plant are therapeutically active. In Ayurvedic system of medicine, the roots are regarded as bitter, tonic, acrid, styptic, galactagogue, aphrodisiac, immunomodulator, cooling, diuretic. Both the fresh leaves and barks are used to treat dysentery, tumors, diarrhoea, wounds and intestinal ulcer. The leaves, fruits and flowers of this plant are astringent, mucilaginous and demulcentand are used in treating gonorrhoea, leprosy and to promote parturition [1].

Palghar district is rich in terms of home to various medicinal plants. Triumfetta Rhomboidea is reported to possess antibacterial, diuretic, analgesic and anti-inflammatory activity [2]. Generally, all medicines whether they are synthetic or of plant origin, should fulfill the basic requirement of being safe and effective. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility [3]. Cytotoxicity tests are useful to screen chemicals for their intrinsic and relative toxicities. This helps in determining the potential toxic or harmful effect of such compounds to human health that may occur inadvertently during use [4].

Principle of colorimetric assays is the measurement of a biochemical marker to evaluate metabolic activity of the cells. Reagents used in colorimetric assays develop a colour in response to the viability of cells, allowing the colorimetric measurement of cell viability via spectrophotometer. Colorimetric assays are applicable for adherent or suspended cell lines, easy to perform, and comparably economical [5].

SRB (Sulforhodamine B) assay is a rapid and sensitive colorimetric method for measuring the drug-induced cytotoxicity in both attached and suspension cell cultures. SRB is a bright pink aminoxanthene dye with two sulfonic groups. Under mildly acidic conditions, SRB binds to protein basic amino acid residues in TCA-fixed (trichloroacetic acid) cells to provide a sensitive index of cellular protein. SRB assay is also used to evaluate colony formation and colony extinction. The SRB assay is simple, fast, and sensitive. It provided good linearity with

cell number, permitted the use of saturating dye concentrations, is less sensitive to environmental fluctuations, is independent of intermediary metabolism, and provided a fixed end point that is not require a time-sensitive measurement of initial reaction velocity. Reproducibility of this assay is high [5].

The purpose of this research work is to analyze the toxicity for Triumfetta Rhomboidea plant.

Sample Preparation:

The samples were extracted in methanol for 2 days at 10 g/100ml. The extract was filtered and dried on hot water bath for 5 hours in petri dishes. The petri dishes were kept in oven at 500C till the extracts were completed dried. The samples for toxicity were prepared in DMSO at 100 mg/ml and were further diluted in the DMEM media at 1 mg/ml-0.0325 mg/ml.

Toxicity on 3T3 cell line:

Methodology:

Viability:

In a 96 well plate, 0.5 x 104 cell/ well (3T3 Mice Fibroblast cell line) was added and the plate was incubated at 370C / 5% CO2 for 24 hours. After incubation media was removed and 100 µl of different concentrations of test and standard with and without LPS were added to the plate. The plate was incubated for further 24 hours at 370C / 5% CO2.SRB staining was performed to estimate cyto-toxic effect of extract and standard. For SRB assay, the cells were fixed using 40µL of cold 10% TCA for 1 hour at 40C.After 1 hour, TCA was removed and the plates were washed four times with distilled water and the plates were dried completely with an aid of tissue papers. After the plates were completely dried, 40 µL of 0.04% SRB dye was added and the plate was incubated for 30 mins. After 30 mins, the SRB dye was aspirated and the plate was washed with 1% Acetic acid glacial to remove the unbound dye. Tris base (10mM, pH 10.5) was added to the plate at 100 µL to extract the bound dye. The plate was read at 510-530 nm. The cell viability was calculated using the formula As X *100/Ac where As is absorbance of sample and Ac is the absorbance of the control.

Results:

Conc	Average	Percent Viability	Standard Deviation	IC 50 value
0.0625	0.249	119.551	0.011	> 1mg/ml
0.125	0.256	123.077	0.005	
0.25	0.247	118.590	0.008	
0.5	0.221	106.410	0.007	
1.0	0.192	92.468	0.015	

Conclusions:

Cell viability tests are important for plant analysis as they provide the number of viable cells and dead cells by the end of the experiment. The present work was carried out to identify cytotoxic effects of Triumfetta Rhomboidea as the plant possess various medicinal properties. This plant is abundantly found in the region of Saphale, Maharashtra. The plant is composed of various active constituents which are used in herbal medicines and therefore it becomes necessary to analyze its cytotoxic effects. The plant extract was subjected to cell viability tests through SRB assays. The obtained results of the plant extract did not show any toxic effects till 1mg/ml. Therefore, composition below 1mg/ml can be subjected to medicinal preparations.

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