EVALUATION OF ANTI PROLIFERATIVE POTENTIAL OF *SOLANUM VIRGINIANUM* HUMAN HEPATOCELLULAR CARCINOMA CELL LINE

RUNNING TITLE: Evaluation of anti proliferative potential of *solanum virginianum* human hepatocellular carcinoma cell line

ABSTRACT

INTRODUCTION

Solanum virginianum commonly known as wild eggplant or nightshade plant, is a prickly herb found in most of the parts of Asia and Australia of the world. It is used by the local people as folk medicines in treating throat infections and other inflammatory problems. Various phytoconstituents have been found, the major constituent is alkaloid. It has a vital role in various traditional as well as medicinal uses for curing internal and external physiological disorders. To determine the evaluation of anti proliferation potential of *Solanum virginianum* human hepatocellular carcinoma cell line.

MATERIALS AND METHOD

The effect of *solanum virginianum* hepG2 cell viability was measured by MTT assay. Briefly, the cells (1×10^4 cells per *mL*) were seeded in a 96 well microtiter plate (100 per well) with replications. Treatment was conducted for 24 hr with different concentrations (50, 100, 150, 200, 250, 300 µM) of solanum virginianum. Cell morphological changes were observed in phase contrast microscopy.

RESULT

Cells were treated with *solanum virginianum* at higher concentration (50, 75, 100, 200, 300 and 400 µM) for 24 h, and cell viability was evaluated by MTT assay. Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

CONCLUSION

From the results, the extracts were cytotoxic to the hepG2 cells at (60µg/*mL*) concentration and incubation period. However more research is needed to understand the mechanisms of cytotoxicity of the plants.

KEYWORDS: Solanum virginianum, anti proliferative, carcinoma cell line, human hepatocellular

INTRODUCTION

InVitro human cell line models are widely used for cancer pharmacogenomic studies to predict clinical response, to assist generate pharmacogenomic hypotheses for further testing, and to assist in identifying novel mechanisms related to variation in drug response(1). Among cell line model systems, immortalized cell lines like Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) are used most frequently to check the effect of ger*mL*ine genetic variation on drug efficacy and toxicity(2). Another model, especially in cancer research, uses neoplastic cell lines like the NCI-60 panel. These models are used mainly to see the effect of somatic alterations on response to anticancer therapy. While these cell line model systems are very useful for initial screening, results from integrated analyses of multiple omics data and drug response phenotypes using cell line model systems still must be confirmed by functional validation and mechanistic studies, also as validation studies using clinical samples(3). Future models might include the employment of patient-specific inducible pluripotent stem cells and the incorporation of 3D culture which could further optimize in vitro cell line models to improve their predictive validity(4–6).

Cell culture and cell lines have assumed a crucial role in studying physiological, pathophysiological, and differentiation processes of specific cells(2). It allows the examination of stepwise alterations within the structure, biology, and genetic makeup of the cell under controlled environments(3,7). This is often especially valuable for complex tissues, like the pancreas, which consists of varied cell types, where *Invivo* examination of individual cells is difficult, if not impossible(3). The acute difficulties within the isolation and purification of individual epithelial cells from complex tissues by maintaining their native characteristics have hampered our understanding of their physiological, biological, growth, and differentiation characteristics(4,5).

Medicinal plant *Solanum virginianum* L belongs to the family solanaceae, commonly known as yellow berried nightshade(8). It is coined by different vernacular names like in Marathi called bhui ringani, Sanskrit Kantakari etc. It is frequently distributed in Asia (Saudi Arabia, Yemen, Afghanistan, Iran, China, Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Vietnam, Indonesia, Malaysia are native countries) and is adventives in Egypt(9,10). In India it's recorded tropical, subtropical and every one four realms(9). Frequently it's been considered as a weed plant but in Ayurveda and folklore medicine since past there are major

reports in literature about its other potentials. The morphologically *Solanum virginianum* is a prickly diffuse bright green perennial herb, somewhat woody at the bottom while the stem is zigzag, and branches are numerous(9–11).

Solanum xanthocarpum is employed by the local people as folk medicines in treating throat infections and other inflammatory problems(12). The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic and aphrodisiac activities. The fruit paste is applied externally to the affected area for treating pimples and swellings(12–14). The various parts of the plant are reputed in indigenous Hindu Medicine to have high medicinal value in various diseases like cough,asthma,fever,heart disease etc. The plant extract of S. xanthocarpum also possesses insecticidal and molluscicidal properties(12,13). Its fruits are eaten as an anthelmintic and for indigestion. Its root is an expectorant, used in Ayurvedic medicine for cough, asthma, and chest pain(15). It has been utilized in Ayurveda for a spread of therapeutic purposes.

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors. It ranks fifth in frequency worldwide among all malignancies and causes 1 million deaths annually, yet its incidence is increasing steadily in various countries(2). Epidemiology studies showed that primary liver cancer is the second mortality in China and it accounts for 53% of all liver cancer deaths worldwide(16). Though with great development in diagnosis and therapy, the prognosis of patients with HCC remains dismal for its high rate of metastasis and recurrence. For patients in advanced stages, the Median survival is less than 6 mo, no matter what kinds of therapy were managed(16,17). So it's urgent to further explore the mechanism of HCC occurrence, progress and metastasis. HCC cell lines are powerful tools. Until now, ten human HCC cell lines have been established, and every cell line has its own characteristics and offers convenience for various experiments(16–19). Stability, homogeneity and straightforward culture are important parameters permanently in cell lines. Previously our team had conducted numerous studies which include clinical trials(20), in vitro studies and case reports.(21) (22) (23) (24) (25) (26) (27) (28) (29) (30) (31) (32) (33) (34)(21) Aim of the study is to determine the evaluation of anti proliferation potential of *Solanum virginianum* human hepatocellular carcinoma cell line.

MATERIAL AND METHOD

PREPARATION OF THE HERBAL EXTRACT

Leaf powder of *Solanum virginianum* obtained from IMPCOPS (Chennai, India) was used for the present study. About 50 g of *Solanum virginianum* powder was soaked in 500 *mL* of aqueous solution and kept at room temperature up to 3 days undisturbed. Then the solution was filtered with crude filter paper followed by whatman paper. Fine filtrate was subjected to rota evaporation. After that 3g of the material was obtained. The total aqueous extract was concentrated in a vacuum evaporate and immediately stored at 4 degree Celsius.

CELL CULTURE REAGENTS

DMEM, Growth medium (DMEM with 10% FBS), Phosphate buffered saline (PBS; pH 7.4), Trypsin-EDTA versus glucose solution, 0.89% physiological saline.Human hepatocellular carcinoma (HepG2) cell line was procured from the National centre for cell science (NCCS, Pune), India. The cells were grown in T255 culture flasks containing DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using trypsin-EDTA solution.

PASSAGING OF CELL

The medium from the culture flask was aspirated. The flask was rinsed with 2*mL* of PBS and aspirated again quickly. 1.5 *mL* of trypsin - EDTA solution was added and incubated at 37 degree celsius for about 2 minutes until cells started detaching. As soon as the cells were detached, transfer pipette into a 15 *mL* falcon tube and it was centrifuged at 1000 rpm for 5 minutes. The medium was carefully aspirated off and care was taken not to put the pipette tip in the bottom of the tube, where the cells were pelted. The cells were gently resuspended in the DMEM medium with 10% FBS by pipetting up and down 5-8 times gently. From the cell suspension, a drop was placed on the edge of the cover slip of the neubauer haemocytometer. The drop was led to run under the cover slip by capillary action. Care was taken not to force the liquid and the entry of the air bubble was avoided. Then the cells from E1, E2, E3, E4 and E5 squares were counted under a microscope. The cells were then gently resuspended and transferred to sterile T 75 culture flasks and the volume of medium was made upto 10 *mL* with growth medium per flask.

TESTING VIABILITY OF CELLS

The viability of HepG2 cells was assessed by trypan blue exclusion test perry et al., (1997). 100µl of trypan blue solution wasmixedwith100µlofcellscontainedinthemediumand incubated for 5 min at 37°C . The cells were then washed thrice with saline and 10µl of this suspension was placed in a haemocytometer and viewed under a microscope. The unstained cells represented the viable cells whereas the damaged cells were stained. The number of stained cells was counted and the percentage of viable cells was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{No. of unstained cells}}{\text{Total no. of cells}} \times 100$$

The viability of the cells was found to be between 90-95%.

CELL PROLIFERATION ASSAY

The proliferation of HepG2 was assessed by MTT assay safadi et al.m (2003). The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (Mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert

internalised tetrazolium salt to formazan crystals, which are purple in colour. Then the cells are lysed using a 20% SDS solution, which releases the formazan crystal. These crystals are solubilised by DMF present in the solubilizer. The colour developed is then determined in an ELISA reader at 620 nm.

PROCEDURE

HepG2 cells were placed in 24 well plates at a concentration of 5 x 10^4 cells/well 24 hours after plating. Cells were washed twice with 500 µl of serum-free medium and starved by incubating the cells in serum - free medium for an hour at 37°C. After starvation, cells were treated with ION of different concentrations for 24 hours. At the end of treatment, the medium from control and ION treated cells were discarded and 500 µl of MTT containing DMEM (0.5 mg/*mL*) was added to each well. The cells were then incubated for 4 h at 37°C in the CO_2 incubator The MTT containing medium was then discarded and the cells were washed with 1 x PBS (1 *mL*).The crystals were then dissolved by adding 500 µl of solubilisation solution and this was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a microplate reader at 620 nm. The OD of each sample was then compared with the control OD & the graph was plotted.

MORPHOLOGICAL STUDY:

Based on MTT assay, we selected the low and high doses of *Solanum virginianum* for further studies. The characterisation of morphological changes in HepG2 cells treated with low and high doses of *Solanum virginianum* compared to their respective controls were observed under phase contrast microscope.

STATISTICAL ANALYSIS

All data obtained were analysed by student - t -test using MS Excel, represented as mean±SD for six animals in each group. The results were compared statistically (SPSS/10 Software package; SPSS Inc., Chicago, IL, USA) using one way ANOVA . Post-hoc testing was performed for inter comparison using the LSD. In all tests, the level of statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

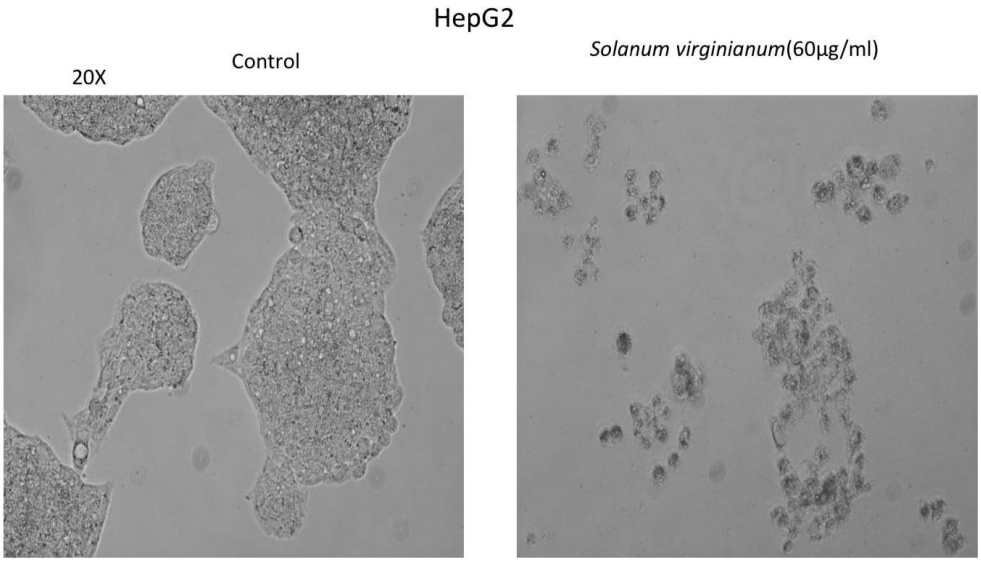

Figure1: Formation of purple formazan crystals in *Solanum virginianum* treated hepatocellular cells (Observed by phase contrast microscopy).

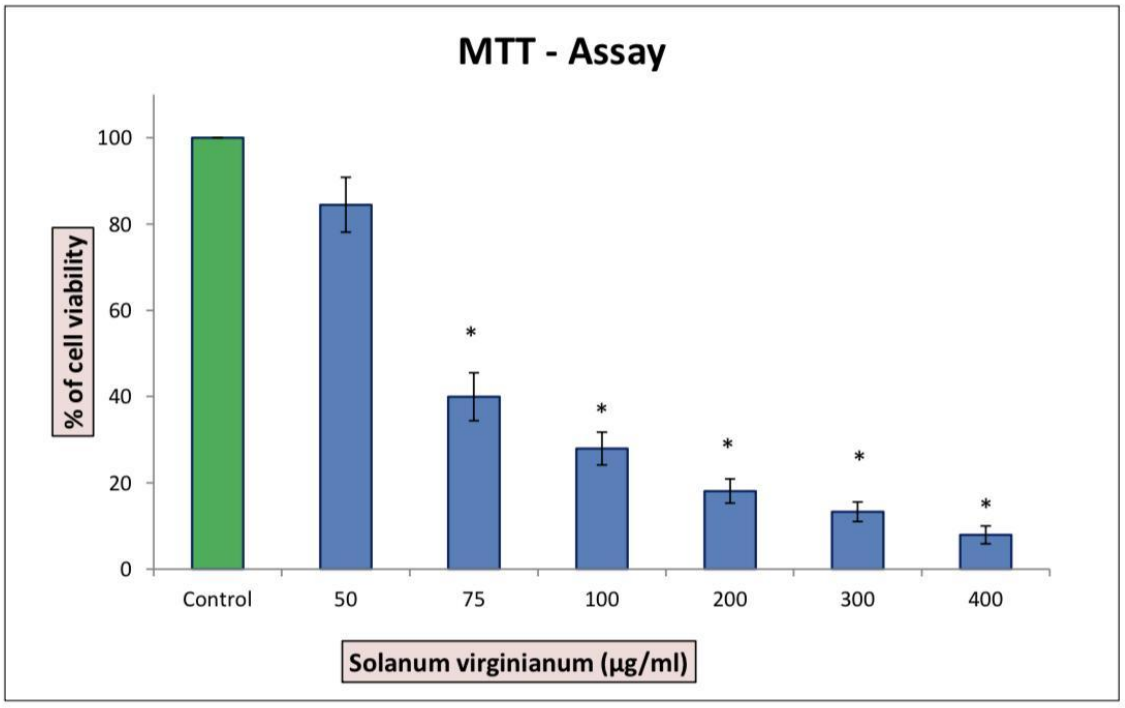

Figure 2: Evaluation of anticancer activity of *Solanum virginianum* on human hepatocellular carcinoma by MTT assay. At a concentration of 60µg/*mL*, the percentage of the cell viability is

50% (IC 50). * represents statistical significance between control versus treatment group was $p<0.04$ at 60µg/*mL*, with $p<0.05$ level using Student's - Newman-Keuls test, statistically significant.

Currently, there's a rise in the number of safe nutraceutical drugs. The standard pharmaceutical plants are an important economic source of raw materials for the drug industry. Meanwhile, a number of untamed plants revealed antioxidants, antimicrobial, anti-inflammatory, and antitumor properties. Medicinal plant research within the family of solanaceae has revealed anti-cancer efficacy in preclinical models. The asterid dicot genus, belonging to the tribe of the solanaceae family, Frequently it's been considered as a weed plant but in Ayurveda and folklore medicine since past there are major reports in literature about its other potentials. *Solanum virginianum* has been used formerly as a traditional treatment and it contains various classes of compounds as flavonoids, phenols, glucosinolates, cardiac glycosides, alkaloids, steroids, terpenes, and coumarins. which are consequently, among the foremost potential mediators that might be accustomed treat chronic diseases. These phytochemicals are characterized by atom scavenging skills; therefore, they have been recommended as anti-inflammatory, antibacterial, and antitumor activities. Natural products are a great source of the latest therapeutic agents which are broadly explored as anti-cancer drugs from plants, marine organisms, and microorganisms. Numerous researches have shown that natural components are suitable to prevent and heal cancer by pointing to its vital hallmarks. Some natural herbal drugs also are established to treat HCC. Within the existing study, MTT assay showed that *Solanum virginianum* significantly reduced the cell viability and proliferation of HCC cell line in an exceedingly concentration-dependent manner, suggesting that *Solanum virginianum* could represent a unique candidate agent for curing HCC. Human tumor-derived cell lines are widely used as models for studying cancer biology. They're very useful to know mechanisms that drive resistance and sensitivity to anticancer compounds, particularly when access to tissue samples is prescribed, as in HCC that noninvasive imaging has replaced biopsy for diagnosis. Over the past decade, several large-scale pharmacogenomic studies in neoplastic cell lines, including NCI-60, neoplastic cell Line Encyclopedia, and GDSC (Genomics of Drug Sensitivity in Cancer), have proven their value for biomarker discovery furthermore on uncover mechanism of drug action and determine molecular contexts related to specific tumor vulnerabilities. Although these programs have provided a wealth of publically available data for the scientific community, HCC cell lines were underrepresented in these different datasets. Given the molecular heterogeneity of HCC, the analysis of an outsized panel of cell lines recapitulating HCC diversity is also more informative and will help to translate in vitro pharmacogenomics findings into clinical application.Our team has extensive knowledge and research experience that has translate into high quality publications(35–39),(40),(41),(42),(43),(44),(45),((37,46,47),(48–52) ,(53),(54)

CONCLUSION

To the best of our knowledge, this is the first study illustrating the anti-apoptotic effect of *Solanum virginianum* on the HepG2 cell line(55-(64). It showed effective anticancer activity. Based on the above-mentioned results, this study suggests that *Solanum virginianum* may be a candidate agent for the treatment of human HCC.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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