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Original Article

CYTOTOXIC ACTIVITY OF METHANOLIC EXTRACTS OF *SOLANUM ERIANTHUM* D. DON

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ABSTRACT

Objective: The study was aimed to evaluate cytotoxic activity of the methanolic extracts of *Solanum erianthum* on HeLa, HepG-2 and MCF-7 cell lines.

Methods: The percentage viability of the cell lines were carried out using trypan blue dye exclusion method and the cytotoxicity of the extracts was evaluated by MTT assay.

Result: *Solanum erianthum* leaf and fruit extract showed significant cytotoxic activity on the tested cell lines with IC_{50} values ranging from 111.67 to 385 µg mL^{-1}. Fruit extract showed potent cytotoxic activity on MCF-7 (IC_{50} 111.67 µgmL^{-1}), followed by leaf extract (IC_{50} 150.50 µgmL^{-1})on HeLa cell line using MTT assay. Cell viability assay showed that fruit extract was very active on MCF-7 and leaf extract was effective against HeLa.

Conclusion: The experiment performed reveals that *Solanum erianthum* can be used in development of anticancer drug.

Keywords: *Solanum erianthum*, Cytotoxic activity, MTT assay, Trypan blue assay, HeLa, HepG-2, MCF-7.

INTRODUCTION

Plants have been a source of medicine for thousands of years and phytochemicals continue to play an essential role in medicine. The use of medicinal plant extracts for the treatment of human diseases is an ancient practice and this has greatly increased in recent years [1]. Cancer is one of the most life-threatening diseases with more than 100 different types. Due to lack of effective drugs, expensive cost of chemotherapeutic agents and their side effects, cancer can be a cause of death [2]. For a long time, plants have been used in the treatment of cancer which contain many effective anticancer agents [3]. This forms the basis for new drug discovery based on traditional plant usage. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them [4-5].

Members belonging to *Solanaceae* are known to be a rich in active secondary metabolites. Numerous species of *Solanum* are known to possess a number of biological activities including antimycotic, antiviral, molluscicidal, antimalarial, teratogenic and cytotoxic properties [6-10]. *Solanum erianthum* is a medicinal plant whose leaves have been extensively used for treating leucorrhoea, piles, hemorrhoids, scrofula, headache, vertigo, digestive troubles and for wound healing purposes [11]. It is reported to have diuretic, purgative properties and active in the treatment of venereal disease and leprosy [12]. This plant is known to contain alkaloids solanine or solasodine, which is the nitrogen analogue of diosgenin and pharmacologically accepted as its alternative [13-14]. *Solanum erianthum* possess antimicrobial property and its fruits are rich in sesquiterpenoids [15-16], and known to have anti cancer, immunosuppressive and anti-inflammatory property [17-18]. Literatures are available on cytotoxic activity of the genus *Solanum*. The *in vitro* cytotoxic property of the crude extract of leaves and berries of *S. aculeastrum* was tested against HeLa and MCF-7 cell line [19]. Sanjay *et al* (2009) studied the anticancer activity of the fruits of *Solanum nigrum* on the HeLa cell line [20]. Leaves of *Solanum anguivi* showed cytotoxic activity against HepG-2 and MCF-7 cell lines [21]. *Solanum xanthocarpum* was also reported having anticancerous property on Hep-2 cell line [22].

However, *Solanum erianthum* is yet to be subjected for its cytotoxic tests to ascertain its cytotoxic activities with potential clinical application.

MATERIALS AND METHODS

Plant material

The plant specimen was collected from outskirts of Bangalore city and was identified as *Solanum erianthum* D. Don. (Syn *S. verbascifolium* non L) and subsequently authenticated by Regional Research Institute, Central Council for Research in Siddha and Ayurveda with the voucher specimen no RRCBI-4865. The voucher specimen is deposited in the herbarium of the same institute.

Extraction

The field grown plants was washed with tap water followed by distilled water to remove the adhering dust particles. After blotting the samples, were air dried in shade. The dried plant materials were ground to fine powder and stored in clean air tight containers. 30 g of sample was placed in the soxhlet and the sample was run by using 250 mL of methanol at 40 °C for the extraction of bioactive compound. All the extracts were dried in vacuum rotary evaporator at 40 °C under reduced pressure. Each of these extracts was weighed and stored at 4°C for further use.

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Trypan blue, Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium

HeLa (Human Epithelial carcinoma cell line), HepG-2 (Human Hepatocellular liver carcinoma cell line) and MCF-7 (Human breast cancer cell line) was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10 % inactivated Fetal Bovine Serum (FBS), penicillin (100 IUmL^{-1}), streptomycin (100 µgmL^{-1}) and amphotericin B (5 µgmL^{-1}) in a humidified atmosphere of 5 % CO_2 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 cm^2 culture flasks (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, each weighed extracts were separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2 % inactivated FBS to obtain a stock solution of 1 $mgmL^{-1}$ concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

MTT Assay

The ability of the cells to survive a toxic treatment has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3- (4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue colored product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used [23].

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5cells/mL using DMEM containing 10 % FBS. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off; wash the monolayer once with medium and 100 µL of different test concentrations of test drugs was added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 ^oC for 3 days in 5 % CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 ^oC in 5 % CO_2 atmosphere. The supernatant was removed and 100 µL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}) values is generated from the dose-response curves for each cell line.

%Growth Inhibition = 100-[Mean OD of individual test group / Mean OD of control group] x 100

Trypan blue dye exclusion technique

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay was based on the assumption that the dead cells will take the dye and viable cells won't [24].

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5cells/mL using DMEM medium containing 10 % FBS. To each of 40 mm Petri dish, 1 mL of the diluted cell suspension (approximately 100,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was removed, washed the monolayer once with medium and 1 mL of different test concentrations of test drug was added on to the partial monolayer in culture dishes. The dishes were then incubated at 37°C for 3 days in 5 % CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were removed and cells were trypsinized. The cells were suspended in PBS and centrifuged to separate cell pellet, re suspended in 1 mL of fresh medium and performed dye exclusion test i. e. equal quantity of the drug treated cells and trypan blue (0.4 %) were mixed and left for a minute. It was then loaded in a haemocytometer and viable and non-viable count was recorded within two minutes. The percentage growth inhibition was calculated and IC_{50} value is generated from the dose-response curves for each cell line.

%Growth Inhibition = 100 – [Total Cells – Dead Cells / Total Cells] x 100.

RESULTS

In the present study, we applied the MTT test to evaluate the cytotoxic effect of methanolic extracts of *S. erianthum* on HeLa, HepG-2 and MCF-7 cell line. The cancer cells were exposed to increasing concentrations (0.1-1 $mgmL^{-1}$) of methanolic for 24 h. The MTT assays with corresponding IC_{50} are summarized in Table 1. The results showed that the methanolic leaf and fruit extract of *S. erianthum* posses potent cytotoxic activity. The lower IC_{50} values represent the higher potency of the extracts to inhibit the growth of cells. Fruit extract exhibited significant cytotoxic activity on MCF-7 by MTT assay with an IC_{50} of 111.67 μgmL^{-1}, followed by leaf extract with an IC_{50} of 150.50 μgmL^{-1}on HeLa cell line.

Percentage cell viability of both of cells was carried out by using trypan blue dye exclusion technique. The extracts showed different antiproliferative effect with respect to extract concentration. There were different inhibitions produced by different concentrations at 24 h incubation. Cell viability assay showed that fruit extract was active on MCF-7 with IC_{50} value 118.67 μgmL^{-1} and leaf extract was effective against HeLa with IC_{50} value of 235 μgmL^{-1} (Table 1). IC_{50} values for both the assay ranged between 111.67 to 385 μgmL^{-1}. Root and stem extracts did not show anticancer activity under the experimental conditions tested.

DISCUSSION

Cervical and breast cancers are the most dreaded cancer types among women [25]. Liver cancer has also become one of the major types of cancer with high mortality and is not responsive to the current cytotoxic agents used in chemotherapy and existing effective therapies have harmful side effects [26]. A vast variety of naturally occurring substances have been shown to protect against experimental carcinogenesis [27]. Natural and some synthetic compounds can prevent, suppress, or reverse the progression of cancer. Although tumors have traditionally been treated with chemotherapeutic agents, the advents of compounds which prevent malignancies represent an emerging field and offer new options [28].

In the present study, the fruit and leaf extracts of *S. erianthum* showed inhibitory effect on cancer cell lines in the *in vitro* studies. It was effective in suppressing the proliferation of the three most relevant cancerous cell lines, HeLa, MCF-7 and HepG-2 in a dose-dependent pattern. Cytotoxic activity of *S. erianthum* possessed better anticancerous activity than *S. nigrum* against HeLa [20]. The ethyl acetate extract of the *S. anguivi* leaf revealed much higher IC_{50} value against HepG-2 and MCF-7 [21]. Similarly MTT assay of *S. xanthocarpum* leaf showed an anti proliferative activity on HEP-2 cells at a higher concentration [22]. However, the methanolic fruit extracts of *S. aculeastrum* was effective against HeLa and MCF-7 cell lines at a very low concentration [19]. To be a good drug candidate, the IC_{50} value of such agent should be sufficiently low to avoid any possible unspecific effects. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bioguided studies if it exerts an IC_{50} value < 30 μgmL^{-1}[29]. In this study, we have focused our interest on crude plant extracts, the cytotoxic activity could be due to the presence of active compounds that could probably have inhibitory effects on the cancer cell lines.

The phytochemical analysis of *S. erianthum* revealed the presence of phenols and flavonoids [15] and the cytotoxic activity of leaf and fruit is attributed to the presence of these compounds. Several studies have shown high cytotoxic and anticancer activity of flavonoids [30]. Flavonoids reduce breast cancer cell proliferation [31], by inhibiting cell growth, protein kinase activities, and induction of apoptosis [32]. Many phenolic compounds were found to have anticancerous or anticarcinogenic activity [33]. Phenolic compounds inhibit different cell cycles at different cell phases and have indirect effect on cell cycle arrest and subsequently induce apoptosis [34-35]. However, plants belonging to the *Solanaceae* family have been reported to contain complex glycosides and saponins [36], which may be responsible for the observed activity. In conclusion, the *in vitro* study identified cytotoxic potential of *S. erianthum* leaf and fruit. These findings provide additional evidence that this plant might indeed be a basis for alternative therapy and also constitute a potential source for the discovery of novel drugs to combat these diseases.

Table 1: Cytotoxic activity (IC_{50}) of methanolic extracts of *S. erianthum*

Sample	MTT assay (µgmL^{-1})			Trypan blue assay (µgmL^{-1})		
	HeLa	HepG-2	MCF-7	HeLa	HepG-2	MCF-7
Leaf	150.50±25.2*	225±15.2*	187.50±12*	235.54±14*	385.09±19.1*	245.31±18.4*
Fruit	142.28±7.3*	189.33±5.5*	111.67±7.3*	156.63±8.3*	207.67±5.0*	111.27±1.7*
Stem	>1000	>1000	>1000	>1000	>1000	>1000
Root	>1000	>1000	>1000	>1000	>1000	>1000

n=18, *p ≤ 0.05

CONFLICT OF INTERESTS

Declared None

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