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Evaluation of cytotoxicity of aqueous extract of *Euphorbia hirta* using human colon adenocarcinoma and vero cell line

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Abstract

Euphorbia hirta Linn. belonging to family Euphorbiaceae. Traditionally it is used in the treatment of tumors, fever, asthma, bronchitis, chest congestion, etc. Objective of the present investigation was to evaluate cytotoxicity of aqueous extract of *E. hirta* using human colon adenocarcinoma (HCT-15) and vero cell line. MTT assay was used to evaluate cytotoxicity of aqueous extract of *E. hirta* using HCT-15 and vero cell line. Doxorubicin was used as standard reference drug. The concentrations of extract and doxorubicin at range 0.05-1000 µg/ml were considered. Results of the present study was revealed that the 50% cell growth inhibition (IC50) of aqueous extract of *E. hirta* and doxorubicin was 510.66 µg/ml and 460.13µg/ml using the HCT-15 cell line and 1363.65 µg/ml and 2392.71µg/ml using Vero cell line respectively. The cytotoxic effect of aqueous extract of *E. hirta* may be due to the presence of previously reported chemical compounds by earlier scientists in the plant. This plant can be used as good source of cytotoxic compounds. Chemical analysis of this extract is in under pipeline. First time author have reported these studies on *E. hirta* using HCT-15 and vero cell line by MTT assay. Further series of studies will required for validating these results by using *in-vivo* pharmacological experiments. The work can be further expanded for detail mechanistic based studies by doing Tunnal, Caspcase and flow cytometry assays.

Keywords: *E. hirta,* Doxorubicin, HCT-15 and Vero cell line

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Introduction

The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management. Chemoprevention involves the use of pharmacological, dietary bio-factors, phytochemicals and even whole plant extracts to prevent, arrest, or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies. [1]

Euphorbia hirta L. (Sp. Pl. (1753) 454.) is an erect or ascending annual with hairy stems, 6-20 inch or 15-50 cm high and opposite, elliptic oblong or obovate-lanceolate leaves. Seeds are 0.8 mm long, ovoid-trigonous, slightly transversely rugose, light reddish brown. Plant is common in waste ground throughout the hotter parts of India and Ceylon-Most tropical and subtropical countries. The plant is chiefly used in the affections of childhood, in worms, bowel complaints and cough. It is sometimes prescribed in gonorrhea. A decoction is used in asthma and chronic bronchial affections. Leaves are used in curing sores, their juice is sometimes squeezed into eyes to cure eye trouble. The root is given by the sandals to allay vomiting and the plant to nursing mothers when the supply of milk is deficient or fails. Juice of the plant is given in dysentery, colic, aphthae and milk applied to destroy warts. It has a depressant action on the heart and respiration and relaxes the bronchioles. The leaves are eaten as vegetable that

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contain moisture (78.14%), protein (4.65%), ether extract (1.71%), ash (3.15%), vitamin-C (44.32 mg/100 gm).[2-4] *E. hirta* has reported to have antiamoebic[5] , antiasthmatic[6], antibacterial[7,8], antidiarrheal[9,10], antifungal[11], anti-inflammatory[12,13], antimalarial[14], antioxidant[15], antitumor[16-18], cytotoxicity[19], etc. activities. Moreover, various chemical constituents have been identified in *E. hirta* which includes, quercetin, triacontane, jambulol, euphosterol, gallic, melissic, palmitic, oleic and linoleic acids, l-inositol, xanthorhamnin[4], afzelin (I), quercitrin (II), and myricitrin (III)[20], rutin (IV), quercitin (V), euphorbin-A (VI), euphorbin-B (VII), euphorbin-C (VIII), euphorbin-D (IX), 2,4,6-tri-*O*-galloyl-β-D-glucose, 1,3,4,6-tetra-*O*-galloyl-β-D-glucose, kaempferol, gallic acid, and protocatechuic acid.[21,22] *E. hirta* also contains β-amyrin, 24-methylenecycloartenol, β-sitosterol, heptacosane, nonacosane[23], shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose and chtolphenolic acid.[24] Toxicological information on *E. hirta* is not available so far. Therefore, the effort to evaluate cytotoxicity of aqueous extract of *Euphorbia hirta* using HCT-15 and vero cell line was undertaken.

Materials and Methods

Chemicals

MTT dye powder (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (Serva Electrophoresis, New Delhi), DMSO cell culture grade (Bioworld), Amphotericin-B (Himedia), Penicillin and Streptomycin solution stabilized (Sigma), EDTA (MP Biomedicals), DPBS / modified 1X (Dulbecoo's Phosphate Buffer Saline without Ca^{+} and Mg^{+}) (Himedia), FBS (Fetal Bovine Serum, South American origin, 500 ml) (Sigma, Mumbai). All other chemicals used for this experimental work were of analytical grade.

Collection and authentication of plant

Whole plant of *E. hirta* were collected from the local region of Amalner town and authenticated by taxonomist at Centre for Post-graduate studies and Research in Botany, Pratap College, Amalner, Jalgaon, Maharashtra.

Preparation of extract

The whole plant were dried, powdered and defatted with petroleum ether followed by methanol and distilled water by using percolation method of extraction. The extracts get concentrated and preserved in an airtight container under refrigeration for further evaluation.

Procurement and maintenance of cell lines

HCT-15 and Vero cell line were procured from National Centre for Cell Science, Pune. Stock cells of these cell lines were cultured in Dulbecco's Modified Eagles Media (DMEM), supplemented with 10% FBS (Fetal bovine serum). Along with media cells were also supplemented with 5 % HBSS, penicillin, streptomycin and amphotericin-B, in a humidified atmosphere of 5 % CO_2 at 37 °C until confluence reached. The cells were dissociated with 0.2 % trypsin, 0.02 % EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 cm^2 tissue culture flasks, then in 75 cm^2 and 150 cm^2 tissue culture flask. The cytotoxicity experiment was carried out in 96 microtitre well plates.

Cytotoxicity assay

Assay protocol described by Mossmann with some modifications was adopted.[25-35] Briefly, cell lines in exponential growth phase were washed, trypsinized and re-suspended in complete culture media. Cells were seeded at a concentration of 2×10^4 cells / well in 96 well microtitre plate and incubated for 24 hrs during which a partial monolayer forms. The cells were then exposed to various concentrations of the test compounds and standard drug doxorubicin, (i.e. Conc. 1000–0.05 µg/ml). Control wells were received only maintenance medium. The plate was incubated at 37 °C in humidified incubator with 5 % CO_2 and 75 % Relative Humidity for a period of 24 hrs. At the end of 24 hrs, 10 µl MTT labeling mixture was added and incubates for 4 hrs. The absorbance was measured using microplate ELISA reader at wavelength 590 nm. The percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula,

$$\% \text{ viability} = (A_T - A_B) / (A_C - A_B) \times 100$$

Where, A_T = Absorbance of treated cells (drug); A_B = Absorbance of blank (only media) and A_C = Absorbance of control (untreated)

% cell growth inhibition / % cytotoxicity = 100 – %

cell survival

All the values of percent cell growth inhibition was replicates of three independent observations (n=3). The 50% cell growth inhibition (IC_{50}) was determined by interpolating concentration (X-axis) Vs % cell inhibition (Y-axis) by linear regression equation using Microsoft Excel, 2007, Microsoft Corporation, USA application.

Results

Results of the present study was revealed that the 50% cell growth inhibition (IC_{50}) of aqueous extract of *E. hirta* and doxorubicin was 510.66 µg/ml and 460.13µg/ml using the HCT-15 cell line (Fig. 1 & 2) and 1363.65 µg/ml and 2392.71µg/ml using vero cell line respectively (Fig. 3 & 4). The aqueous extract of *E. hirta* was found to be effective cytotoxic. It was safe against normal vero cell line with respect to the concentrations used in the studies.

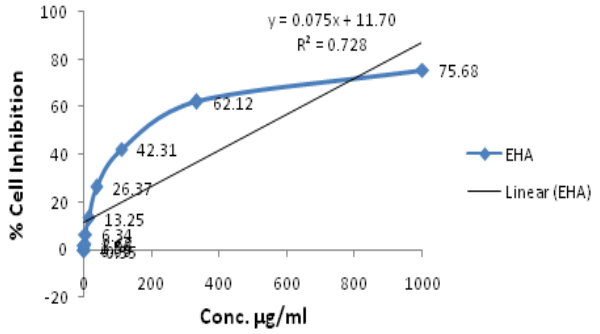


Figure 1. % cell inhibition of aqueous extract of *E. hirta* on HCT-15 cell line.

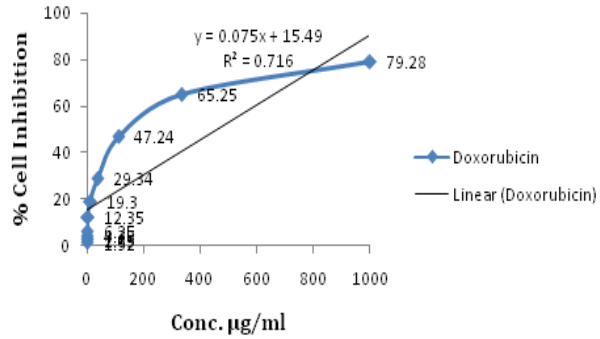


Figure 2. % cell inhibition of doxorubicin on HCT-15 cell line.

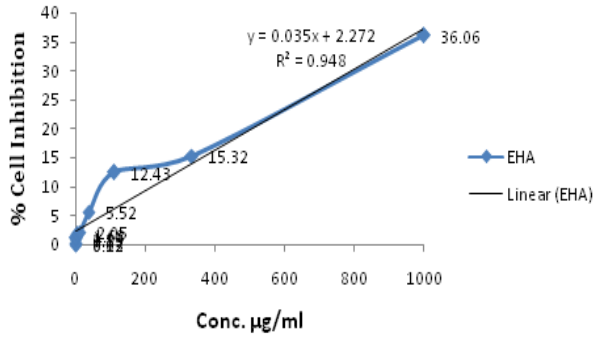


Figure 3. % cell inhibition of aqueous extract of *E. hirta* on vero cell line.

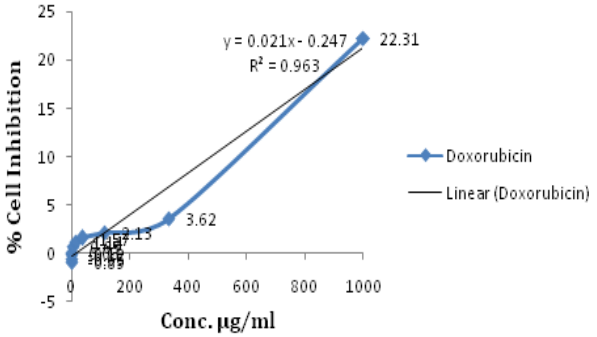


Figure 4. % cell inhibition of doxorubicin on vero cell line.

Discussion and conclusion

Carcinoma of the breast, colon and prostate are highly prevalent malignancies in the Western nations and accounts for approximately half of the total cancer-related deaths among men and women.[36] Approximately, 60% of the anticancer drugs currently have been isolated from natural products.[37] Cytotoxicity assays (CTAs) are performed to predict potential toxicity, using cultured cells which may be normal or transformed cells. These tests normally involve short term exposure of cultured cells to test substances, to detect how basal or specialized cell functions may be affected by the substance, prior to performing safety studies in whole organisms. It can also provide insight towards the carcinogenic and genotoxic dispositions of herb-derived compounds and extracts. The ability of a plant extract to inhibit cellular growth and viability can also be ascertained as an indication of its toxicity. Assessment parameters for cytotoxic effects include inhibition of cell proliferation, cell viability markers (metabolic and membrane), morphologic and

intracellular differentiation markers.[38] The cytotoxic effect of aqueous extract of *E. hirta* may be due to the presence of previously reported chemical compounds by earlier scientists in the plant. This plant can be used as good source of cytotoxic compounds. Chemical analysis of this extract is in under pipeline. First time author have reported these studies on *E. hirta* using HCT-15 and vero cell line by MTT assay. Further series of studies will required for validating these results by using *in-vivo* pharmacological experiments. The work can be further expanded for detail mechanistic based studies by doing Tunnal, Caspcase and flow cytometry assays.

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Conflict of Interest

None Declared

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