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RESEARCH ARTICLE

Invitro Cytotoxic Effect of Methanolic Extract from *Eclipta prostrata L.* against Human Colorectal Adenocarcinoma Cell Line

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ABSTRACT

Colorectal cancer is the third leading affected in men after lung and prostate cancer tumours and in women after lung and breast cancers. Current food habitation as potential risk factors for the growth of colorectal cancer include lack of physical activity, alcohol consumption, smoking, a low fiber and high fat nutrition, obesity and inadequate fruit and vegetable usage. *Eclipta prostrata (L).* is commonly found on roadsides and waste lands. It is annual herbaceous plant have a long history of traditional values; medicines use which is various part of the world especially in tropical, subtropical regional widely. This herbs already found the curative properties and has been utilized as analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too. Present study to find the in vitro cytotoxic effect of methanolic extract against the Human Colorectal Adenocarcinoma cells. Cancer cells were treated with different concentration of extract and incubated for 24hours and the cytotoxic effect was observed in microscopy and cell viability was determined by 2,5-diphenyl-2H-tetrazolium bromide assay shows half maximal inhibitory concentration shows 62.44 µg/ml. The nuclear 4'6-diamidino-2-phenylindole staining was performed and confirm the existence of apoptosis morphological changes of the cells were studied. Besides, this study to continue the identification of specific metabolites from methanolic extract of *Eclipta prostrata (L.)* recommended for the discovery of potential anti-proliferative and anticancer compounds.

Keywords: *Eclipta prostrata (L.)*, Colorectal Cancer, Methanol Extract, Cytotoxicity

Introduction

Eclipta prostrata (L). known as Bhringaraj, an annual herbaceous plant has been traditionally used as a liver tonic in Ayurveda and hair growth promote and maintenance of pigment[1,2]. This plant act as a potential memory modulator and also have a medicinal value of analgesic, antiseptic, antiviral, antibacterial, antioxidant, antihaemorrhagic and anti-hyperglycemic activity reported by The Indian Pharmacopoeia Govt of India Publication[3]. *E.prostrata (L.)* plant is widely used in different regions of India, China, Nepal, Brazil for the treatment of skin problems, hepatic problems, respiratory problems, gastrointestinal problems and other symptoms such as hair loss, hair whitening, wounds remedies[3,4]. The leaves are used to treat snakebite and catarrh[5,6]. Currently, pharmaceutical industries invest immense capital in seeking promising medicinal herbs with novel chemical compounds which could be used as disease therapies[7]. Several plants are used to manage the above treatments. Particularly earlier phytochemical studies on this plant revealed the presence of triterpenes, thiophene derivatives, steroids[8], polypeptides, polyacetylenes, coumestons and flavonoids[9].

Information on chemical composition of these plants can be generated for further advanced research work[10], which may include the isolation of these chemicals and their subsequent use in the development of more efficient and safer of drug preparation. This viable source of therapeutic agents from nature might not be unconnected to the structural diversity inherent in a million species of plants and microbes but still remains beyond the comprehension of human[11]. Drugs currently in use as anticancer have over 60% of their origin from natural products[12]. These research study focused on invitro cytotoxicity effect against Human Colorectal Adenocarcinoma (HT29) cancer cells in order to evaluate the potential for the further development of single product in multiple therapeutic purpose.

Materials and Methods

E. prostrata (L.) was collected in and around the Rathinam institution, Echanari, Coimbatore District, Tamil Nadu, India and identified specimen voucher had deposited in our institution (Voucher No. RCASEP2019008)

Preparation of Extract

The *E.prostrata (L.)* required quantity of the whole plant powder was weighed and extracted for soxhlation with methanol. The extract was further removing the solvent from Rotary vacuum evaporator (Perfit India). The residues obtained were dissolved with DMSO stored in a freezer -20°C until further test.

Cell Culture

The human colon adenocarcinoma (HT-29) cell line was maintained at 37°C in a humidified atmosphere (90%) containing 5% CO2 and then cultured in DMEM with 10% (v/v) FBS, 100 units/ml penicillin and 100µg/ml streptomycin. The different concentration of plant extract used incubate the cells were seeded overnight.

Cell Viability Assay

The modified MTT assay used to determine cell viability of HT29 cells [13]. In brief, the cells ($1x10^4$ cells/well) were seeded in 96-well plates and exposed to the indicated concentration of the extract for 24 hrs. The sample of minimum 3.185 µg/ml and maximum 1000 µg/ml concentrations. The sample were dissolved in DMSO and further diluted with cell culture medium. Adjusted with 1% DMSO as a final concentration of the medium in all the test reaction including blank. After the treatment, 5 mg/ml of MTT solution were added and incubated for 3hrs at 37°C in a dark place. The absorbance was measured at a wavelength of 540 nm with ELISA plate Analyser (Robonik- India). The cell viability by MTT assay was calculated as a percentage of the control value using the following formula: % cell viability = A540 of treated cells/A540 of control cells x 100.

Morphological assessment by 4'6-diamidino-2-phenylindole staining

The 4'6-diamidino-2-phenylindole (DAPI) staining was carried out according to the method described by Kntayya *et. al* [14], HT29 cells were grown on sterile glass slides overnight and treated for 48hrs with plant extract at IC50 concentration. The cells were incubated for 24 hrs in a humidified atmosphere of 5% CO2 at 37°C. At the end of the incubation, cells were fixed with 4% Formaldehyde and stained using DAPI in PBS described by Pakizehkar *et. al*[15] for 15 seconds at room temperature in a dark condition. Finally, the stained cells were washed with PBS and the nuclear morphological changes were viewed at tenfold magnification using inverted microscope.

Statistical Analysis

All values are expressed as mean ± S.D. The test of significance between two control and test was estimated by Student's t test. GraphPad Prism version 4.00 used to perform the One-way ANOVA with Tukey's post test. The level of statistical significance accepted was considered as $p<0.05$.

Results

The HT-29 cells were treated with different concentration of the methanolic extract of plant *E. prostrata (L.)* for 24hrs. The MTT assay used to determine the cell viability. The dose of the methanolic extract inducing IC_{50} of HT-29 Human colorectal cell are presented in Table.1. The concentration of extract in different ratios treated cell with control Figure 1A. The IC_{50} of extract shows 62.44 µg/ml respectively. The phase contrast microscopy used to visualize the cellular morphology of HT-29 cell line. As shown in Fig1.B the treatment of cells in plant extract induces the significant cellular morphological changes like apoptotic bodies, cell debris, cell shrinkage and detached cells.

Table 1: *in vitro anticancer effect of samples on Human colon cancer cell lines (HT-29)*

S.no	Concentration	Dilution	% cell viability
			Sample SD
1	1000	Neat	35.12±1.23
2	500	1:1	42.88±1.89
3	250	1:2	46.17±1.89
4	125	1:4	53.43±0.87
5	100	1:8	57.90±1.23
6	62.5	1:6	64.17±0.87
7	31.25	1:32	77.66±1.32
8	15.625	1:64	86.65±1.89
9	10	1:128	89.90±1.65
10	3.185	1:256	93.52±1.89
11	Cell control	-	100

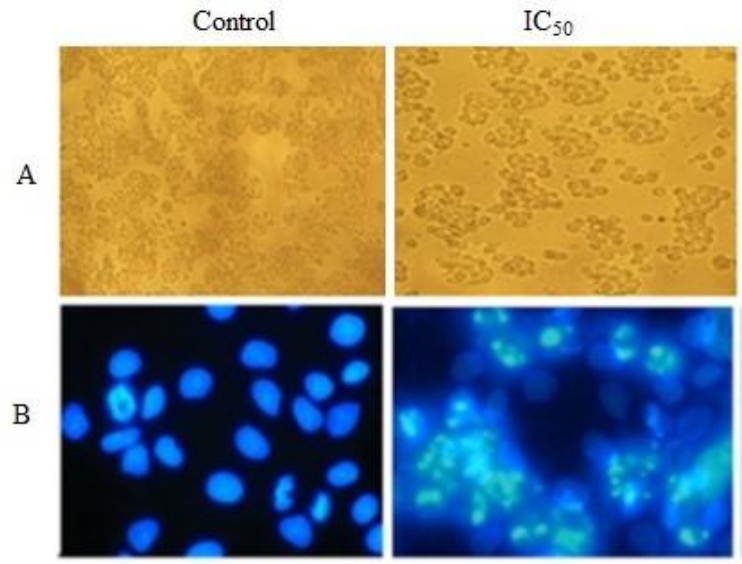

***Figure 1 A** - Cytotoxicity Effect of plant extracts on HT-29 cell lines, Control and IC50 Concentration. **B**- Morphological characteristics associated with apoptosis, treatment the cells were fixed stained with DAPI.*

Discussion

The compounds obtained from *E.prostrata (L.)* using both *Invitro* and *Invivo* models due to the wide range of medicinal values and applications have been performed regarding the biological activities[16,17,18]. This plant have been different extraction method used to studied the antimicrobial property against *Staphyloccus sp. Salmonella sp. Pseudomonas sp. Shigella sp.* and *E.coli*[19]. Antibacterial activity and anti-oxidant properties shown in ethanol extract[20] and also, they reported analgesic activity in mice [21] moreover to find the lowered the lipid levels in rats[22]. Fresh green leaves-based study has shown a protective effect on carbon tetrachloride induced liver damage in guinea pigs[23]. Aqueous and ethanol extract of the plant have shown neuropharmacological activities in rat[24]. The tumor growth inhibition of breast cancer cells which selective regulation of Hsp60 cell by *invivo*[25]. Various compounds isolated from aerial parts of plant were evaluated cytotoxicity activities against human ovarian cancer cell lines assessed by MTT assay and the IC50 values of more than 10 compounds were less than 100μM and much more effective for HepG2 cells[26, 27]. The anti-invasion screening using human colon adenocarcinoma (HT-29) cells, in the present showed significance inhibition by treatment with the methanolic extract. Further need to investigate the *invivo* methods to find the inhibition of cell migration. Since the plant has been shown to induce the white blood cell count, phagocytic index and titrate the antibody in mice[28, 29], this plant a promising agent for inhibiting tumor progression and enhancement of immune function in cancer patients, because *E.prostrata (L)* have immunostimulating effect combined with anti-metastatic and anti-angiogenic effects.

Conclusion

Most of the experiment were performed by *invivo* method using rat's model. The clinical significance is not well studied. Further research needs to take human and animal models along with the active compounds isolated this plant may lead to the discovery of potent therapeutic agents. The clinical studies are necessary to evaluate the safety and efficacy of *E.prostrata (L.)* in future for designed properly. Moreover, to concentrate the product-based development of traditional medicine for promoting activities.

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