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PHYTO-PHARMACOLOGICAL AND IN-VITRO ANTICANCER ACTIVITY OF *WEDELIA CHINESIS* AGAINST HT-29 (HUMAN COLON CANCER)

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ABSTRACT

In this study, we evaluated anticancer activity of ethanol extract of *Wedelia chinesis (W.chinesis)* against HT-29 (Human colon cancer) Cytotoxicity of *W.chinesis* was investigated using MTT assay. Doxorubicin was used as the positive control. MTT assay showed activity against the tested cell line. The cytotoxicity activities were expressed as percentage of cell viability. Cytotoxicity of ethanolic extract of *W.chinesis* against HT-29 cell line was measured and the IC50 value of ethanol extract of *W.chinesis* was 57.28±0.152 µg/mL µg/ml. IC50 value of Doxorubicin (standard) was 11.30±0.20µg/mL. Morphological alteration of HT-29 cells lines upon exposure using *W.chinesis* extract was observed under phase contrast microscope. The cells indicated the most prominent effects after exposure to the *W.chinesis* extract. The present findings confirmed cytotoxic effect of ethanol extract of *W.chinesis* HT-29 (Human colon cancer)

KEYWORDS: anticancer, cell lines, Medicinal plants, herbs, cytotoxicity, flavanoids, polyphenolic compounds.

1. INTRODUCTION

The vinca alkaloids (vincristine, vinblastine and vindesine) and the podophylotoxin derivatives (etoposide and teniposide) are examples of clinically active plant products.[1] The goal of screening medicinal plant is to search for excellent anticancer agent avertable to human malignancies. In defiance of astonishing advances in modern medicine, such as surgery, radiotherapy, chemotherapy, and hormone therapy, cancer disease remains a worldwide health problem, hence leading the research area for new alternate approach. The nature is a huge valuable contributor of potential source for chemotherapeutic agents and it has recently been reviewed. Tumor cell grow rapidly and these uncontrolled growth is a common property of tumour cells. Medicinal plants have the property to control the growth of tumor cells. Hence analysis of cancer cell growth inhibiting mechanism is very useful to understand the anticancer property of medicinal plants. Analysis and identification of novel molecules with anti-tumor activity is useful for the development of anti-cancer drugs.[2] The ethanol extract of *Wedelia chinesis (W.chinesis*) insinuated good biological activity earlier including anticancer activity. The current study was undertaken with the objective to rationalize the cytotoxicity effect of *W.chinesis* ethanol extract on HT-29 cell lines in accordance to the observable changes of cell morphology upon exposure to the extract.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The entire plant of *Wedelia chinesis (*Osbeck*)* Merr and *Wedelia calendulaceae* (L.) Less were collected from Karyavattom campus on April 2015. Plant material was air dried in the laboratory for 5 days at room temperature followed by oven drying at 40oC then grinded to powder form using an electric mill. The powdered sample was kept in an air tight container until required. Preparation of the different extracts of *Wedelia chinesis* (Burm.f.) Merr. was done by soxhlet extraction.

2.2. Cytotoxicity Screening

2.2.1. Cell Lines

In this study cancer cell line HT-29 (Human colon cancer) was obtained from National Center for Cell Sciences (NCCS), Pune. Human breast adenocarcinoma HT-29 cells were derived from breast cancer which was obtained from American Type Culture Collection (ATCC: Manassas, VA). HT-29 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine (2 raM), penicillin (100 units/mL) and

streptomycin (100 µg/mL). The cells were cultured at 37°C in a humidified 5% CO_2 incubator.

2.1.2. Cytotoxicity assay

The extract of *W.chinesis* was tested for *in vitro* cytotoxicity, using HT-29 (Human colon cancer) cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[3] Briefly, 100 µL of media (RMPI 1640) was added into each of the 96-well plates from row B to row G (triplicate). Then, 100 µL of diluted plant extract or fractions were added in row A and row *B. Starting* from row B the 200 µL of solution (100 µL drug + 100 µL media) were mixed and 100 µl from row B were added into next row (row C) by using micropipette and a serial dilution was done up to row G. Finally, excessive 100 µL from row G were discarded. The final volume for each well was 100 µL. The cultured HT-29 (Human colon cancer) were harvested by trypsinization, pooled in a 50 mL vial. Then, the cells were plated at a density of 1×106 cells/mL cells/well (100 µL) into 96-well micro-titer plates from row B to row G. Finaly, 200 µL of cells (Vero/MCF-7) were added in row H as a control. Each sample was replicated 3 times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/mL) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under a microscope. Flowingly, the medium together with MTT (190 µL) were aspirated off the wells, DMSO (100 µL) was added and the plates shaken for 5 min. The absorbance for each well was measured at 540 nm in a micro-titre plate reader[3] and the percentage cell viability (CV) was calculated manually using the formula:

$$CV = \frac{\text{Average abs of duplicate drug wells}}{\text{Average abs of control wells}} \times 100\%$$

A dose-response curve was plotted to enable the calculation of the concentrations that kill 50% of the HT-29 (Human colon cancer) (IC_{50}).

2.2.3. Morphological analysis

Morphological observation of cell treated with *W.chinesis* extract from cytotoxicity study was done to determine the changes induced by the extracts. Changes such as shrinking of the cells, membrane blebbing, ballooning, chromatin condensation, formation of apoptotic bodies were observed in predicting the apoptotic mechanism for cell death. Meanwhile, vacuolations of the cytoplasm and formation of double membrane vesicle containing organelles were assessed for authophagic cell death.

3. RESULTS

3.1. Proliferative effects of HT-29 and Vero cells

The effect of anticancer from *W.chinesis* on HT-29 (Human colon cancer) cell lines was evaluated thorugh micro-culture tetrazolium assay (MTT). The multiple concentrations of ethanolic extract from *W.chinesis* was used and effective doses were calculated from dose-response curve. Results of the cytotoxicity evaluation against HT-29 (Human colon cancer) cell line of the *W.chinesis* extract are shown in figure. Ethanol extract of *E. guineensis* exhibited significant activity against the HT-29 (Human colon cancer) cell line with an IC_{50} value of 57.28±0.152 µg/mL. IC50 value of Doxorubicin (standard) was 11.30±0.20 µg/mL. On treatment with *W.chinesis* extract, the HT-29 cells showed an increased rate of cell death at a lower concentration of the extract.

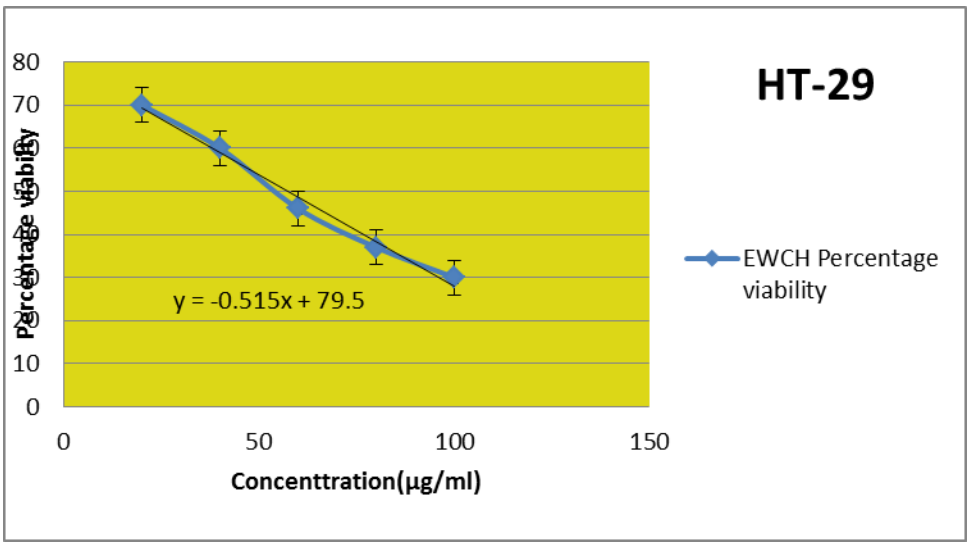

Figure 1: Toxicity effects of the *W.chinesis* methanol extract against cancer HT-29 (Human colon cancer) **after 24 hours of incubation.**

3.2. Evaluation on morphological changes upon treatment with extracts

Morphological alteration of HT-29 (Human colon cancer) cells lines upon exposure using *W.chinesis* extract was observed under phase contrast microscope. The cells indicated the most prominent effects after exposure to the *W.chinesis* extract. The microscopic observations revealed the *W.chinesis* extract to be having outstanding effect on treated HT-29 (Human colon cancer) cells untreated cells (Figure 2). The number of dead cells increased correspondingly with concentration increment of the extract treatment in regard to observation. At high extract concentration, enlargement of the cells was conspicuosly observed. 40%-50% of the cells showed membrane blebbing (demonstrated with small protrusions of the membrane) and ballooning were apparent in the cells. The presence of apoptotic bodies could also be seen in the extract treated cells (Figure 2). Cells also showed extensive vacuolation in the cell cytoplasm, indicating autophagy like mechanism of cell death. Autophagosome like structures were clearly seen in the cells treated with *W.chinesis* extract (Figure 2). At highest concentration (100 µg/mL) the cells became rounder, shrunken and showed signs of detachment from the surface of the wells denoting cell death.

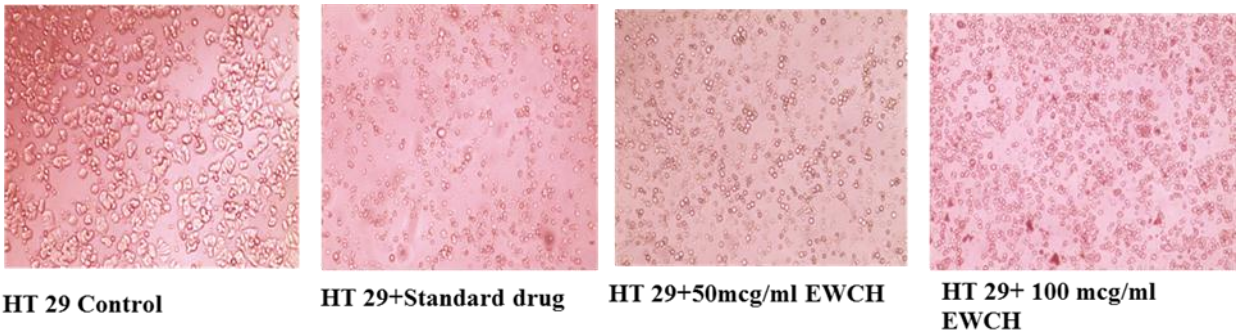

Figure 2: Morphological changes of the (A) HT-29 after *W.chinesis* ethanol extract treatment.

4. DISCUSSION

The contribution of new and novel products from potential bioactive plants or their extracts for disease treatment and prevention is still vast, despite the overshadowing by recent synthetic chemistry as a method of drug discoveries and drug productions.[5] Moreover, plant derived drugs like vinblastine, vincristine, taxol, and camptothecin had lead to greatest extend within the vicinity of antitumor upon where, the drugs were reported to improvise the chemotherapy of some cancers.[6] Plants contain almost unlimited capacity to generate compounds that fascinates researchers in the quest for new and novel chemotherapeutics.[7] The persistency search for new anticancer compounds in plant medicines and traditional foods is a realistic and promising strategy for its prevention.[8] Numerous compounds found in plants with anticancer properties are such as alkaloids, phenylpropanoids, and terpenoids.[9],[10]

Therefore, in this study *W.chinesis* extract was evaluated as new anticancer agent by using MTT assays. Plants used in folk and traditional medicines have been accepted as leads for therapeutic drug development in modern medicine. *W.chinesis* was chosen for this study because it us having chemical constituents with anticancer activity.[11] Hence this study the cytotoxicity was evaluated *in vitro*. Studies have observed the presence of a large number of bioactive compounds in the ethanolic extracts of this plant such as polyphenolic compounds and flavonoids which exhibit various biological activities.[11,17] These compounds are present in a number of food items and hold great potential as drug candidates due to their safety, low toxicity and wide acceptance amongst the public.

The present study also demonstrated the cytotoxicity indices as a measure of percentage cell mortality calculated by MTT assay in HT-29 (Human colon cancer) cells in a dose dependent manner at the end of 24 hours incubation with extract. HT-29 (Human colon cancer) was used as the test system in this study which was prompted by the requirement of more effective treatment for the increasing incidence of Human colon cancer worldwide. The extract was able to inhibit the proliferation of the cancer cell at (57.28±0.152µg/mL). However a crude extract with IC_{50} less than 100 µg/mL is considered highly cytotoxic.[18] The results of the present study showed potent cytotoxic effects on HT-29 (Human colon cancer) cells with *W.chinesis* extract. The morphological effects were more prominent in the acetone extract treated cells showing extensive blebbing and vacuolation suggesting autophagic mechanism of cell death.

The investigation provides evidence for cytotoxicity in MCF-7 which may be due to existing phytochemicals in the extract since *W.chinesis* as mention previously. The sensitivities of cancer cells to cell death by flavanoids[19] are accordance with this finding from previous reports in literature.[20]

This finding suggests that the reduction observed in the viable cells following treatment with *W.chinesis* extract is due to cell death. In conclusion, the present observations provide preliminary data exposing

W.chinesis extract to have potent cytotoxic activity against HT-29 (Human colon cancer) cells. This calls for further studies on the active components for proper assessment of their chemotherapeutic properties as well as their possible development as promising anticancer drugs.

Conflict of interest statement: We declare that we have no conflict of interest.

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