INTRODUCTION

Human colon cancer is one of the leading causes of cancer death in both men and women worldwide and it is a clinically common, highly malignant tumor of the digestive tract.1 Currently, colon cancer is the third most common cancer type in humans, the fourth most common cause of death because of cancer, and the second most common cancer type in terms of the number of individuals living with cancer 5 years after diagnosis worldwide, approximately 694,000 people die from colon cancer annually.2 Diet contributes to 20% to 42% of all human cancers and 50% to 90% of colon cancer.3 The risk factors associated may include a family history of cancer of the colon or rectum, hereditary conditions, such as familial adenomatous polyposis and hereditary non-polyposis colon cancer, a history of ulcerative colitis or Crohn’s disease, personal history of cancer of the colon, rectum, ovary, endometrium, or breast, polyps in the colon or rectum.4 Normal treatment modality includes surgery, radiofrequency ablation, cryosurgery, chemotherapy, radiotherapy, targeted therapy with monoclonal antibodies, angiogenesis inhibitors. This is regardless of widespread, effective measures of preventive screening, and also major advances in treatment options. Due to an increasing rate of mortality associated with cancer and adverse or toxic side effects of cancer chemotherapy and radiation therapy, discovery of new anticancer agents derived from nature has begun especially plants and the screening of medicinal plants as a source of anticancer molecules.5 There is an overwhelming evidence of a preventive/protective role of dietary plant extracts, especially fruits, vegetables, grains, and herbs, against colon cancer. Indeed, dietary intervention is emerging as an alternative to prevent the progression of colon cancer mainly due to its potency and reduced toxicity.6,8 Caralluma fimbriata is an edible succulent cactus and wild medicinal plant in the family Apocynaceae, growing in dry places, used by tribal Indians to suppress hunger and known as “famine food” with no history of adverse effects, which contains pregnane glycosides.7 This plant has been investigated for its myriad biological effects such as antihyperglycaemic and hypolipidaemic, hepatoprotective, antioxidant activity and came out with promising results.4,10 In light of above reports, it is reasonable to assume that this plant could have anticancer potential. Hence, in this study we investigated the cytotoxic potential of C. fimbriata ethanolic leaf extract against human COLO 320 cancer cell lines.

MATERIALS AND METHODS

Reagents and Extract

3-(4,5–dimethyl thiazol–2–yl)–5– diphenyl tetrazolium bromide (MTT), Trypan blue, foetal bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco’s Modified Eagle’s Medium (DMEM), Dimethylsulfoxide (DMSO), Cyclophosphamide were purchased from Sigma Chemical, USA and all other chemical used in this study purchased locally and were of analytical grade. The C. fimbriata ethanolic leaf extract was obtained from Green Chem. Herbal Extracts and Formulations, Bengaluru as gratis.

Cytotoxic Effect of Caralluma fimbriata Against Human Colon Cancer Cells

Shenai Ashwini, Devaraj Ezhilarasan, Roy Anitha*

ABSTRACT

Aim: The present study was designed to examine the cytotoxic effects of ethanolic leaf extract of Caralluma fimbriata in the COLO 320 cell line. Materials and Method: The anti-proliferative effects were evaluated using the MTT assay. The COLO 320 cells were treated with different concentrations of the leaf extract of Caralluma (100 – 300 µg/ml) for 24 h. The cell viability and IC₅₀ was calculated from the cytotoxicity. The morphology of the Caralluma treated cells, control, and positive control were observed under reverse phase inverted microscope. Result: The C. fimbriata ethanolic leaf extract showed dose dependent increase in cytotoxicity in COLO 320 human colon cancer cells. The maximum cytotoxic effect was noticed with maximum dose used in this study i.e., 300 µg with an IC₅₀ value of 233.87 µg. Conclusion: The present study shows that the ethanolic leaf extract of Caralluma fimbriata is capable of reducing cell proliferation by inducing cytotoxicity of COLO 320 cells.

Key words: Colonic cancer, COLO 320 cell line, Caralluma fimbriata, cytotoxicity, MTT Assay
Cell culture
The COLO 320 cells were cultured in DMEM supplemented with 10% of fetal bovine serum and 1% penicillin-streptomycin. Cell cultures were maintained at 37°C in a fully humidified atmosphere containing 5% CO₂.

Cell treatment
*C. fimbriata* was dissolved in 0.1% DMSO (v/v). COLO 320 cells were plated at 1.2 X 10⁴ cells/cm². Twenty-four hours later, cells were fed with fresh expansion culture medium supplemented with different final concentrations of extract (100, 200 and 300 µg/ml) or the corresponding volumes of the vehicle. After 24 h of treatment, cells were collected after 0.05% trypsin application. Cell viability was also evaluated by MTT assay.

MTT assay
The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10⁴ cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the sample (100, 200 & 300 µg/ml) for 24 hours. After the incubation, medium was discarded and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml). After 4 hours, the medium was discarded and 100 µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570 nm in a microtiter plate reader. Cyclophosphamide was used as positive control. The experiment was carried out in triplicate. The percentage cytotoxicity was plotted against different concentration and the IC₅₀ values were determined.

RESULTS

Cytotoxic assay
In the present study, we evaluated the cytotoxic potential of *C. fimbriata* ethanolic leaf extract in COLO 320 cells by MTT assay. MTT assay was employed to assess the number of viable cells which has been adapted to measure the growth modulation of cells *in vitro*. The *in vitro* cytotoxicity assays offers quick, simple and cost-efficient way of testing the toxicity and forms. The COLO 320 cells were treated with different concentrations of the ethanolic leaf extract of *caralluma* (100 – 300 µg/ml) for 24 h. *C. fimbriata* ethanolic leaf extract caused dose dependent increase in cytotoxicity in COLO 320 human colon cancer cells. The maximum cytotoxic effect was noticed with maximum dose used in this study i.e., 300 µg [Figure 1]. The ethanolic leaf extract of *C. fimbriata* demonstrated good antiproliferative activity against COLO 320 cells. The extract showed an inhibitory concentration (IC₅₀) is 233.87 µg [Figure 2].

Cell viability
We noticed that *C. fimbriata* treatment in COLO 320 cells caused dose dependent fall in the cell viability. Cytotoxic activity was determined by cell viability and cell morphology was observed under the inverted microscope [Figure 3]. The ethanolic *C. fimbriata* leaf extract decreased the cell viability of colon cancer cells.

DISCUSSION
Colon cancer is one of the most dangerous forms of cancer, with invasive potential to spread to distinct parts of the body including liver, lung, ovaries and other gastrointestinal organs. Colon cancer is currently treated with 5-flourouracil and oxaliplatin. However, while synthetic chemical anticancer drugs prolong survival, they often have adverse effects and off target actions. Accordingly, there is a need to develop novel drug therapies for colon cancer. Several plant derived compounds have studied for their anticancer effect and came with promising results. Naturaceuticals such as silibinin, quercetin, curcumin and plant extracts such as *Curcuma longa, Moringa oleifera, Camellia sinensis, Dictyopteris undulata* have also been tested for their anticancer effects against colon cancer. In previous studies *C. fimbriata* has been evaluated against several cancer models except colon cancer. Methanolic extract of the *C. fimbriata* reported to exhibit anti lung cancer activity, Priya et al. carried out cell viability and growth inhibition activity of the methanolic extract of *C. fimbriata* against A-549 lung cancer cell lines. In *in vitro* cytotoxicity studies are commonly performed to evaluate the anticancer potentials of a drug or plant extracts. In this study, we noticed cytotoxic potential of *C. fimbriata* in human colon cancer cell line. Studies have reported the presence of phytophenol and flavonoids in the ethanolic extract of *Caralluma fimbriata*. *C. fimbriata* reported for appetite control, antiobesogenic and other metabolic regulations. The appetite suppressing properties of *C. fimbriata* has been attributed to the active component, pregnane...
glycosides. Recently, this pregnane glycoside has been reported to possess anti-proliferative and anticancer effects against breast cancer cells. Currently, interest in polyphenols has been raised due to their reported chemo preventive and/or chemotherapeutic potential. In light of the above reports it is to suggest that the profound cytotoxic effect of *C. fimbriata* ethanolic leaf extract in human colon cells could be due presence of pregnane glycosides. The cytotoxic potential of *C. fimbriata* could be due to an induction of apoptosis. However, further studies are warranted on these lines to understand the molecular mechanism of cytotoxicity inducing potentials of *C. fimbriata*.

**ACKNOWLEDGEMENT**

Authors extend their heart felt thanks to Dr. R. Rajendran, CEO, Green Chem Bangalore for providing *Caralluma fimbriata* as gratis for this study.

**CONFLICTS OF INTEREST**

None to declare

**REFERENCES**


13. Sharma R. Nutraceuticals and nutraceutical suppplementation criteria in cancer:
Cytotoxic effect of Caralluma fimbriata

PICTORIAL ABSTRACT

ABOUT AUTHORS

Ms Anitha Roy has completed her M.Pharm from Dr. Hari Singh Gour Vishwa Vidyalaya, Sagar, MP. She is pursuing for her Ph.D at Saveetha University. She is presently working as Assistant Professor in the Department of Pharmacology at Saveetha Dental College and Hospitals. She has published about 50 papers in reputed journals

Dr. D. Ezhilarasan has completed his Ph.D from Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Chennai, India. Currently, he is working as Assistant Professor in Pharmacology, Saveetha Dental College and Hospitals, Saveetha University. He is an active researcher (https://scholar.google.co.in/citations?user=e4kSKvMAAAAJ&hl=en) and his research interests include cancer biology, chronic liver diseases, pharmacognosy and cell signalling.

Ms Ashwini Shenai is currently doing her Bachelor’s degree in Dental Surgery at Saveetha Dental College and Hospitals, Chennai, India. She is a bright student and has keen interest in academic as well as research activity. This work was carried out as a short term project as part of her graduation programme.