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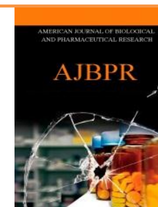

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ANTICANCER AND CYTOTOXICITY ACTIVITIES OF *Clerodendrum inerme* AGAINST HUMAN CERVICAL CARCINOMA AND LIVER CANCER CELL LINES

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ABSTRACT

In recent times the trends in cancer research is shifting towards identifying new medicines from natural resources for management of cancer. Current clinical trends involve the usage of plants as therapeutic agents in a wide range of applications. The present investigation is focused on the plant *Clerodendrum inerme* a hedge plant belongs to the Verbenaceae family, traditionally used to cure many diseases. The aim of the present study is to investigate the anticancer activity of ethanol leaf extracts of *Clerodendrum inerme*. The ethanol leaf extract showed anticancer activity against human cervical carcinoma cell line HeLa and liver cancer (HepG2) cell line with IC_{50} values of 15.6 µg/ml concentration. This study create the awareness of this plant which is having potential activities against cancer which will be boon to the mankind systematic way.

INTRODUCTION

India is known for its rich diversity of medicinal plants and from ancient times these plants were utilized as therapeutic agents. Today's research is mainly focused on medicinal plants because the bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance. The phytochemical constituents, natural bioactive compounds, nutrients and fibers present in medicinal plants, fruits and vegetables defend us from various ailments [1].

Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression.

Several synthetic agents are used to cure the disease but they have high toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer properties. Some studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer compared with chemotherapy or hormonal treatments [2].

Clerodendrum inerme occurs predominantly in the mangrove region of coastal India, exposed to wide range of salinity fluctuations. They have an advantage over other species that lack strategies to deal with salt in the environment and thus are excellent competitors in saline environments [3]. This plant is rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have anticancer properties [4]. Most of the currently used anticancer drugs are highly toxic, expensive, and resistance

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mechanisms pose a significant problem [5-6]. There is a continuing need to identify new drug candidates that are more effective, widely available and less toxic. Plants extracts are an important source of potentially useful compounds for the development of new anticancer drugs. Here we investigated ethanolic leaf extract of *Clerodendrum inerme* against cytotoxic activities in two cancer-derived cell lines.

MATERIALS AND METHODS

Preparation of leaves extract

The selected plant *Clerodendrum inerme* were collected from Thanjavur rural station, Tamilnadu India. Collected plant samples were washed by distilled water (DW) to remove undesirable materials and excess of water was drained off. The leaves were separated from each other and they were sliced into small pieces. The sliced leaves were shade dried for few days. The shade dried leafs were powdered separately by grinding machine and about 5g of powdered leaf of *C.inerme* was taken into clean flat-bottomed glass container and soaked with 200 ml of 95% ethanol. The containers with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The obtained filtrates were rotary evaporated and this dried content was used for anticancer study.

Test chemicals

Growth medium with 10 % FCS, Trypsin (0.25 % + EDTA, 1 mM, in PBS), MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-dephenyltetrazolium bromide, 5 mg/ml, filter sterilized, dissolved in PBS, Dimethyl sulfoxide (DMSO) and Dulbecco's modified eagle's medium (DMEM) were purchased from Himedia (India).

Anticancer activity

Cytotoxic potential of *C. inerme* ethanol leaf extract was tested on human lung adeno carcinoma, human cervical carcinoma cell line. HeLa and liver cancer (HepG2) cell line was purchased from the National Centre for Cell Science (NCCS), Pune, India. The growth medium, Minimum Essential Medium (MEM) was removed after incubation using micropipette. The monolayer of cells was washed twice with MEM without Foetal calf serum (FCS) to remove the dead cells and excess FCS. To the washed cell sheet, 1ml of medium (without FCS) containing defined concentration of the leaf extract in respective wells was added. Each dilution of the compound ranges from 1:1 to 1:128 and they were added to the respective wells of 24 well titre plate. The control well was prepared with cells containing 1ml MEM without any added test sample. The titer plate was incubated at 37^0C in 5% CO_2 environment and observed for cytotoxicity using inverted microscope as well as MTT assay. After incubation, the medium from the wells was carefully removed for MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay. Each well was washed with MEM (without) FCS for 2-3 times and 200µl of MTT (5mg/ml) was added. The plate was incubated for 6 hrs in 5% CO_2 incubator for cytotoxicity. After incubation 1ml of DMSO was added in each well, mixed and left for 45sec. Viable cells present in the medium formed crystals which were dissolved by adding solubilizing reagent Dimethyl sulphoxide (DMSO) that resulted in formation of purple colour. The absorbance of the suspension was measured spectrophotometrically at 540nm by taking DMSO as a blank [7].

The percentage growth inhibition was calculated using following formula,

% cell inhibition= 100-{(At - Ab) / (Ac - Ab)} x100

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

RESULTS AND DISCUSSION

Cancer is one of the most severe health problems in both developing and developed countries, worldwide. Among the most common (lung, stomach, colorectal, liver, breast) types of cancers, lung cancer has continued to be the most common cancer diagnosed in men and breast cancer is the most common cancer diagnosed in women [8]. Plants have long history of use in treatment of cancer. Emerging evidence has demonstrated that many natural products isolated from plant sources possess antitumor properties. Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. Many traditional healers and herbalists have been treating cancer patients for many years using various medicinal plant species [9]. Currently, natural products, especially plant secondary metabolites such as isoprenoids, phenolics and alkaloids, have been demonstrated to be the leading providers of novel anticancer agents so in this regard the present investigation focused on the anticancer activity of medicinal plant *C. inerme.*

HeLa CELL LINE

In the present study HeLa cells were treated with leaf extract at different concentrations, ranging from 3.9 to 500µg/ml, respectively. The IC_{50} value (Half maximal inhibitory concentration) was shown in Table 1. The leaf extract exhibited significant inhibition of cell proliferation which was directly proportion to the test concentration ranging between 3.9 to 500 µg/ml. The highest cytotoxicity of 91.2% was observed at the highest tested concentration of 500µg/ml) and the mean value of

IC_{50} value was observed at the concentration 15.6µg/ml (Fig. 2).The lowest cell inhibition rate was predicted in the lowest concentration taken (3.9µg/ml). Similarly, Asokan*et.al* suggested that methanolic extract of *Saraca indica bark* has significant anti-cancerous activity against HeLa cervical cancer cell lines.[10] Likewise, Ehssan *et.al* reported that fruits of *Solanum nigrum* methanolic extract were tested for its inhibitory effect on HeLa cell line and has significant cytotoxicity effect on HeLa Cell Line in concentrations ranged between 10-0.0196 mg mL^{-1} by using SRB assay and the study also showed that the inhibitory action on HeLa cell line in concentrations ranged between 10-0.0196 mg mL^{-1} by using MTT assay[11].

HepG2 cell line

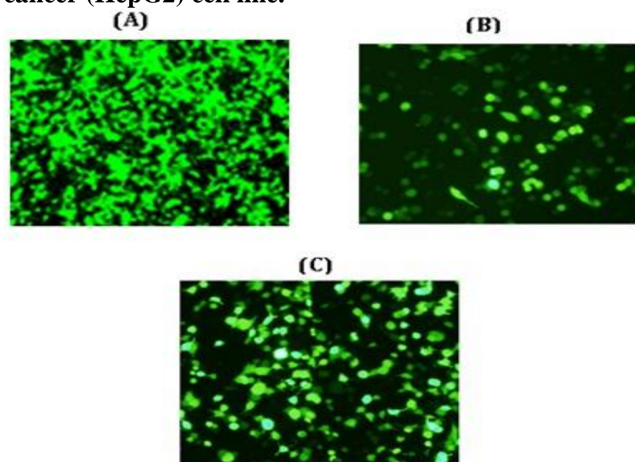
In the present study the cytotoxic effect of the *Clerodendrum inerme* leaf extract on Hep G2 cell lines was examined. Cells were treated with leaf extract at different concentrations ranging from 3.9 to 500µg/ml, respectively. The data of IC_{50} (Half maximal inhibitory concentration) are shown in Table 1. The leaf extract exhibited significant inhibition of cell proliferation of HepG2 cell lines in proportion to its concentration ranging from 3.9 to 500µg/ml. In the highest concentration (500µg/ml) the cell inhibition was in the mean value of 89.7%. IC_{50} value of HEPG2 cell lines was 15.6 µg/ml of extract concentration (Fig. 1) and showed lowest cell inhibition of 40.10% in the lowest concentration taken (3.9µg/ml). Supportively, Machana *et.al* suggested that extract of *C.speciosum* or *P. kesiya* contains fewer polyphenols appeared to have strong anticancer activity in the HepG2 cells [12].

Likewise Lee *et.al* reported that *Terminalia* to exhibit growth suppression against HTC-15 and HepG2cells. *Terminalia chebula* extract has gallic acid and chebulinic acid showed effective growth inhibitory and cytotoxic effects on several human cancer cell lines [13].

Table 1. Cytotoxic activity of ethanolic leaves extract of *Clerodendrum inerme* on HepG2 (liver cancer) cell line and HeLa (Human cervical carcinoma)

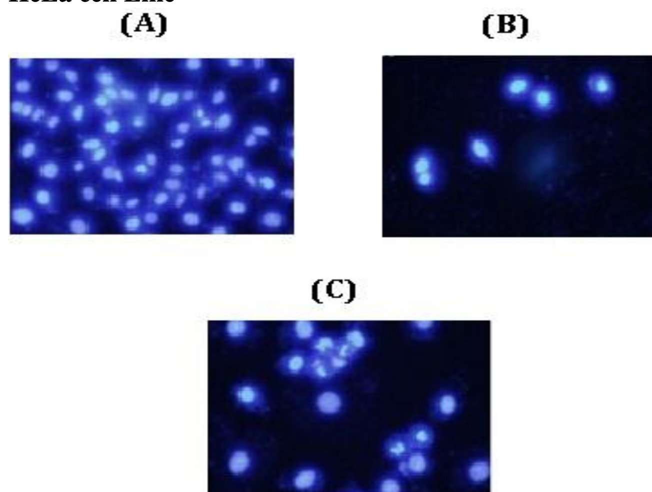
S. No.	Test sample concentration (µg/ml)	0.01% ethanol dilutions	Cell inhibition/ cytotoxicity (%)	
			HepG2	HeLa
1	500	1:1	89.7	91.2
2	250	1:2	71.32	71.41
3	125	1:4	60.14	62.19
4	62.5	1:8	54.53	57.56
5	31.3	1:16	52.23	53.83
6	15.6	1:32	50.43	50.19
7	7.8	1:64	46.01	48.09
8	3.9	1:128	40.10	47.06
9	Cell control	Neat	0.06	0.06

Figure 1. Microscopic examination of cytotoxic effect of Ethanolic leaves extract of *Clerodendrum inerme* on liver cancer (HepG2) cell line.



A- Control B- Ethanolic leaves extract of *C. inerme* (500µg/ml) C- Ethanolic leaves extract of *C. inerme* (15.6µg/ml)

Figure 2. Effect of ethanolic leaves extract of *C. inerme* on HeLa cell Line



A- Control B- Ethanolic leaves extract of *C. inerme* (500µg/ml) C- Ethanolic leaves extract of *C. inerme* (15.6µg/ml)

CONCLUSION

The ethanolic leaf extract of the *C.inerme* showed selective *in vitro* cytotoxicity, active against some human cancer cell lines. Activity depends upon the morphology and mechanism of action plant extract. This plant extract kill the cancer cell lines through activating apoptosis and some through effecting growth regulators. The outcome of the study encourages carrying out further investigation by isolating a particular bioactive compound with anticancer activity so as to design a specific drug for the diseases.

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