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Cyathula prostrata: A potent source of anticancer agent against daltons ascites in Swiss albino mice

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ABSTRACT

Objective: To evaluate the anticancer activity of the methanolic extract of *Cyathula prostrata* in Ehrlich ascites carcinoma (EAC)-bearing mice with methotrexate as positive control in the advanced stage of tumorigenesis. **Methods:** EAC was induced in swiss albino mice by injecting 10^6 cell/mL of tumor cell suspension intraperitoneal. The methanolic extract of *Cyathula prostrata* effect on the tumor cell viability, DNA fragmentation and MTT assay were carried out. **Results:** Methanolic extract attenuated percentage increased in the cell survival time when compared to control group. However, the effect was less than that of methotrexat. Methotrexat and the extracts reversed the tumor-induced alterations in DNA fragmentation and MTT assay. **Conclusions:** The present study suggests that the methanol extract of *Cyathula prostrata* has significant anticancer activity and that is comparable to that of methotrexate.

1. Introduction

Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance, the cancer may be treated. Billions of dollars have been spent on cancer research and yet we do not understand exactly what cancer is. Free radical, one of the major cause for the conversion of normal cell to cancerous cells are generated as a consequences of a number of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer and exposure to a plethora of exogenous chemicals[1]. However, overproduction of free radical and reactive oxygen species would assault on important biological molecules such as DNA, protein or lipid leading to many degenerative diseases, such as cancer, Alzheimer's, arthritis and ischemic reperfusion[2].

Plants have a long history of use in the treatment of cancer. Over 60% of currently used anti-cancer agents are derived in one-way or another from natural sources, including plants, marine organisms and microorganisms[3]. A number of plants have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development[4] due to their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects[5,6]. Hence, the natural products now have been contemplated of exceptional value in the development of effective anticancer agents with minimum host cell toxicity. The present study was carried out to evaluate the antitumor activity of the methanolic extract of the leaves of *Cyathula prostrata* against Ehrlich ascites carcinoma (EAC) in mice.

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2. Materials and methods

2.1. Collection and authentication of Cyathula prostrata

The fresh leaves of *Cyathula prostrata* (Amaranthaceae) were collected in the month of December 2007 from Thrissur district, Kerala, India. Taxonomic authentication was done by Dr.V.S. Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India.

2.2. Preparation of methanolic extract of Cyathula prostrata leaf (MECP)

The leaves material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered leaves (100 g) were extracted with 1 000 mL of methanol using soxhlet apparatus. The extract was filtered through filter paper to get clear filtrate. The filtrate obtained by repeated maceration was evaporated under reduced pressure at 40 ℃ using Rotary evaporator. A dark semisolid material obtained, was stored at −4 ℃, until use.

2.3. Chemicals

The chemicals and solvents used in the study were of highest purity and analytical reagents grade. They were purchased from SD Fine Chem., Himedia and Qualigens, India.

2.4. Experimental animals

Ten-week-old female Swiss albino strain mice weighing (20 ±2) g were used for the study. The mice were procured from the Small Animal's breeding centre of Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The mice were grouped and housed in polyacrylic cages with not more than twelve animals per cage and maintained at temperature of (25±2) ℃; relative humidity of (55±5)%, 14/10 h, dark/light cycle, with free access to feed and water (*ad libitum*). The mice were acclimatized to laboratory conditions for 10 d prior to the commencement of the experiment. The animal care and handling was done according to the regulations of Council Directive CPCSEA No: 659/02/a about Good Laboratory Practice on animal experimentation. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the International Animal Ethics Committee.

2.5. Induction of lymphoma

Dalton's lymphoma ascites (DLA) cells were obtained from Amala Cancer Research Centre, Thrissur, Kerala, India. The cells were maintained *in vivo* in Swiss Albino mice by intraperitoneal transplantation of 1×10^6 cells/mouse. The DLA cells aspirated from the peritoneal cavity of the mice were washed with saline and given intraperitoneally to the experimental animals to develop ascitic tumor.

2.6. Treatment schedule

Sixty Swiss albino mice were divided into five groups (n=12) and given food and water *ad libitum*. All the animals in each groups except Group-Ⅰ received EAC cells (10^6 cell/mL cells/mouse *ip*.). Group-Ⅰ served as normal control and Group-Ⅱ served as EAC control. Group-Ⅲ received extract of *Cyathula prostrata* leaf at a dose of 100 mg/kg *ip*. for eleven consecutive days, respectively. Group-Ⅳ received reference drug methotrexate (3.4 mg/kg *ip*.) for eleven consecutive days. Twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed by cervical dislocation to measure antitumor activity. The antitumor activity of the extract of *Cyathula prostrata* leaf was measured in EAC animals with respect to the following parameters.

2.7. Viable/nonviable tumor cell count

A cell suspension containing approximately 1×10^5 cells/mL was prepared in MEM. 0.5 mL of 0.4% trypan blue solution was transferred to a micro centrifuge tube. To this 0.3 mL of MEM and 0.2 mL of cell suspension were added and mixed thoroughly. The mixture was allowed to stand for 5 min. The suspension was viewed in a homocytometer and looked for viable cells. Viable cell count was determined as per the method of Freshney *et al*[7] by using the following calculations.

Cell count = (Number of cells ×dilution factor)/(Are× thickness of liquid film).

2.8. DNA fragmentation analysis

DNA was purified as described previously[8]. Cells were grown at a density of 1×10^5 cells/mL and treated with various concentrations of *Cyathula prostrata* extract with 250 nM of 13-glucosidase for 0 to 24 h, as described in the figure legends. The resulting purified DNA fragments were subjected to electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining.

2.9. MTT assay

MTT assay was assessed according to the method of Shashi *et al*[9]. Briefly, 200 μL of medium containing 1×10^5 cells were seeded in each well of 96-well microtiter plates. Cells were incubated with different concentrations of ZMS for 48 h at 37 ℃, 5% CO_2 with 98% humidity. The medium was replaced with fresh medium containing 100 μg/mL of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h. The supernatant was aspirated and MTT-formazan crystals were dissolved in 100 μL DMSO. Absorbance was measured at 570 nm.

2.10. Statistical analysis

The results obtained were reported as mean±SD. One way Analysis of Variance (ANOVA) was performed to analyze statistical significance of the data using Agres Statistical Package.

3. Results

Antitumor activity of extract against EAC tumor bearing mice was assessed by the parameters such as cell count (viable and non viable), mean survival time and % increase of life span. The results are shown in Figure 1. The viable cell count were found to be significantly (P<0.001) increased and non-viable cell count was significantly (P<0.001) low in EAC control animals when compared with normal control animals. Administration of methanolic extract at a dose of 100 mg/kg significantly (P<0.05) decreased viable cell count. Furthermore, Figure 2 depicts the agarose gel electrophoresis of DNA from DLA cells treated with *Cyathula prostrata* extract. DNA isolated from DLA cells cultured for 24 h with MECP showed the fragmented laddering pattern characteristics of apoptosis on agarose gel electrophoresis. MECP exhibited a time dependent apoptotic induction in DLA cells and also our present study (Table 1) observed that MECP significantly inhibited the proliferation of DLA cells and its activity in dose as well as time dependent manner. The cytotoxic agent, methotrexate, at the time concentration of 0.68 μg/ml caused cytotoxicity at 62% (24 h), 79% (48 h), 86% (72 h); MECP resulted in toxicity for DLA cells from the concentration of 0.5 mg/mL.

Table 1

Inhibitory effect of methanolic extract of *Cyathula prostrata* on DLA cells *in vitro*.

Groups	Concentration	Growth inhibition (%)		
		24 h	48 h	72 h
MECP (mg/mL)	0.50	22.0±1.2	35.0±1.6	49.0±2.3
	1.00	34.0±1.4	45.0±2.1	56.0±2.8
	1.50	42.0±1.8	57.0±2.8	65.0±3.3
	2.00	51.0±2.4	63.0±3.2	74.0±3.8
Methotrexate(μ g/mL)	0.68	62.0±3.1	79.0±3.9	86.0±4.3

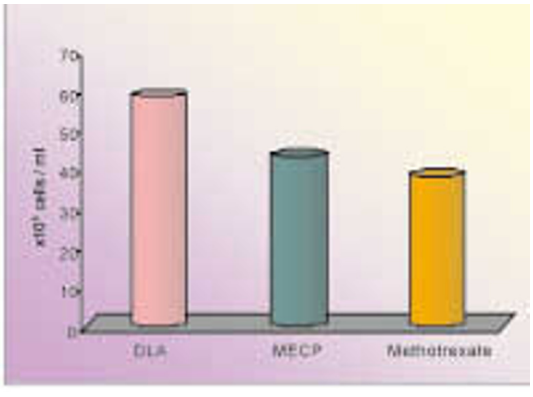

Figure 1. Total cell count and viable cell number.

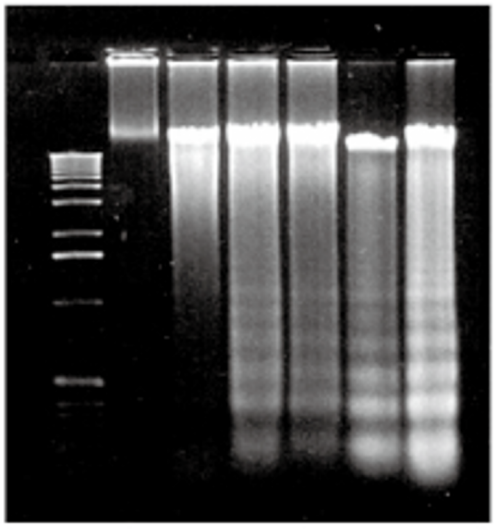

Figure 2. DNA fragmentation analysis.

4. Discussion

Many natural products have served as anticancer agents in treatment and also lead compounds for further research. Many plants are used to treat tumors in Indian traditional system of medicine, but most of the plants have not been scientifically evaluated. The effect of plant extracts as antitumors was widely studied due to their low toxicity and side effects. Cell death is an important process of living system to maintain homeostasis. Apoptosis is a series of genetically controlled events causing cell death, results in the removal of unwanted cells. The characteristics of apoptosis include fragmentation of nuclear DNA cleaved by endonuclease Inducing apoptosis in cancer cells may be useful in the management and therapy of cancer[10].

The ascitic tumor implication induces local inflammatory reaction with an increase in vascular permeability, which results in intense edema formation, cellular migration and a progressive ascitic fluid formation. The ascitic fluid is essential to tumor growth, since it constitutes the direct nutritional source for tumor cells. The viable tumor cell counts in the peritoneum were significantly lower in mice treated with MECP in comparison with the tumor control groups. These results could indicate direct cytotoxic effect of MECP. Similar results where reported in earlier *in vitro* experiments by Dongre *et al*[11].

Many cancer therapeutic agents exhibit their antitumor effects through initiation of apoptosis, it is postulated that they elicit cell death in DLA cells via the induction of apoptosis. Apoptotic cell death is a physiological regulator of tissue growth and homeostasis. One of the most important advances in cancer research in recent years was the recognition that the cell death mostly by apoptosis is

crucially involved in the regulation of tumor formation and determines treatment response. Most anticancer strategies currently used in clinical oncology appear to kill cancer cells, at least in part, by apoptosis and signal transduction pathways.

Cancer chemotherapeutic agents can often provide temporary relief from symptoms, prolongation of life and occasionally complete remission. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damages to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells. Chemopreventive agents comprise diverse groups of compounds with different mechanisms of action with ultimate ability to induce apoptosis. Understanding the modes of action of these compounds should provide useful information for their possible applications in cancer prevention and perhaps in cancer therapy.

Cell cycle modulation by various natural and synthetic agents is gaining widespread attention in recent years. Given that disruption of cell cycle plays a crucial role in cancer progression, its modulation by phytochemicals seems to be a logical approach in control of carcinogenesis. There are a number of herbs that have shown their ability to induce cell cycle arrest and to play an important role in cancer prevention and therapy[12].

Failure of the apoptosis (cell suicide regulation) may cause neoplastic transformation and cancer. The life span of both normal and cancer cells is significantly affected by the rate of apoptosis. Thus, modulating apoptosis may be useful in the management and therapy or prevention of cancer. The ultimate ability of the chemopreventive agents to induce apoptosis may represent a unifying concept for the mechanism of chemoprevention. It is thus considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Certain products from plants are known to induce apoptosis only in neoplastic cells but not in normal cells[10].

Apoptosis or programmed cell death has become of interest as an intervening target in cancer chemoprevention. In the present study, the nuclear DNA of apoptotic cells showed a characteristic laddering pattern of oligonucleosomal fragments. In the setting of carcinogenesis, inhibition of apoptosis has been correlated with tumour promotion. There is abundant evidence that the administration of naturally occurring compounds with antitumour activities triggers the apoptotic death of cancer cells[13]. Induction of apoptosis in cancer cells is recognized as an efficient strategy for cancer chemotherapy and also a reliable marker for the evaluation of potential agents for cancer prevention. In conclusion, the methanolic extract of *Cyathula prostrata* has significantly reduced tumor growth, viability of tumor cells, raising life span as compared with those of EAC control mice. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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