FREE RADICAL SCAVENGING AND ANTICANCER ACTIVITY OF *CLERODENDRON PANICULATUM*.

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Summary

Oxidative stress has been implicated in numerous pathophysiological conditions including cancer. Conventional medical treatment has its own side effects besides the high cost. A simple but effective way of preventing cancer may be to prevent oxidative damage. In the recent years, several antioxidant phenolic compounds have been recognized to have ability to induce apoptosis in various tumour cells of human origin. By virtue of their antioxidant property, they also prevent cell growth by inhibiting the proteins responsible for cell growth. Ethno botanical search reveals use of many traditional herbs in treatment of cancer, which are usually free from side effects, are economical and also easily accessible to humans. However, their mechanism of action is not known. The plant *Clerodendron paniculatum* is a plant widely found in Udupi district of Karnataka, India. The present study was undertaken to evaluate the possible anticancer effects of alcoholic extract of the plant. Although, plants of the same genus were reported to possess potent anticancer activity [6] [7], no reports were available on this plant. The plants of the genus Clerodendron were reported to have antioxidant [10] and anti-inflammatory [8] [9] activities also. The extract was also studied for free radical scavenging potential. Although the extract showed significant antioxidant activity, its anticancer potential was not significant.

Keywords-*Clerodendron paniculatum*, Cisplatin, Anticancer, Liquid tumor, EAC cell lines, antioxidant activity.

Introduction

Cancer is a scourge afflicting mankind from the times immemorial. In spite of the spectacular advances made by medical sciences during the present century, treatment of cancer remains an enigma. Cancer is the second leading cause of death in many of the developed countries and the incidence of cancer is increasing annually. The development of more effective drugs for treating patients with cancer has been a major human endeavor over the past 50 years and the 21st century now promises some dramatic new directions. While improvements in surgery and radiotherapy have had a major impact on cancer treatment, the concept of systemic chemotherapy specific for cancer cells and free of major side effects remains critical goal for the future. However, most cancers can be cured and successfully treated if detected early and most often failure of cancer therapy is due to a delay in diagnosis.

The last couple of decades have brought a massive development of new drugs that affected the integrity of the cell's genetic material, with approximately one new drug entering widespread clinical use every 2 years. Chemotherapy is now employed in the primary treatment or as an adjuvant or important modality in the management of neoplasm. However, the effective doses of most of the chemotherapeutic agents fall in the range of toxic dose. They are highly reactive and are capable of inducing varying degrees of cell destruction and this leads to unpleasant side effects while undergoing treatment. Hence, search for new antitumor agents with high chemotherapeutic value to fight against cancer is a medical priority. The antitumor agents must be able to kill or inactivate tumor cells without damaging normal tissues.

Going through the evolution of the cancer chemotherapeutic agents one could expect to obtain a lead molecule through any one of the drug design methods. One of the potential sources of leads could be the plant kingdom. Plant products have been a source of medicinal agents since time immemorial. From the dawn of civilization, man has been utilizing the important biological properties of various

plants for treatment of different diseases. Even today, plants are the most exclusive source of drugs for the majority of world's population and plant products constitute the 25% of prescribed medicines.

India has a rich heritage of medicinal plants and natural products. The rich herbal resources offer a unique opportunity in our country to evaluate them specifically. The use of plant drugs is increasing in many of the developing countries because modern life saving drugs are beyond the reach of common man and have high toxic potential. Currently there is renewed interest in developing drugs from plants for cancer therapy. Many indigenous plants are being tested against a spectrum of experimental tumors with the perceived advantage of local availability, low cost and less side effects.

The present study was on the plant *Clerodendron paniculatum* belongs to Verbenaceae family. Selection of the plant was based on the fact that other species of the genus Clerodendron viz. *Clerodendon serratum* and *Clerodendron viscosum* showed potent anticancer properties. In view of above facts, the present study has been undertaken on this plant to carry a preliminary investigation for their possible anticancer activity.

Materials and methods

The roots of *Clerodendron paniculatum* was collected from Manipal and Udupi, (Karnataka state) India in the month of October 2006.The plant was authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 558) has been deposited in the herbarium of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Chemicals and instruments

DPPH, ABTS and O-Phenanthroline were procured from Sigma Mumbai, India. Cisplatin injection was procured from Dabur India Ltd, New Delhi.

Preparation of extracts

Alcoholic extract: The powdered plant (2.5kg) was exhaustively extracted by Soxhlet apparatus with 95% ethanol. The total ethanolic extract was then concentrated *in vacuo* to syrupy mass.

Experimental

In vitro anti-oxidant studies

1. Reduction of 1, 1- Diphenyl- 2- Picryl Hydrazyl (DPPH) Free Radical [1].

To 1ml each of various concentrations of ethanolic extract in ethanol, 1ml of solution of DPPH (0.1 mM) was added and incubated at room temperature for 20 min. Absorbance's of the solutions were then measured at 517 nm. Ascorbic acid was used as the standard for comparison. Experiment was performed in triplicate.

2. ABTS radical scavenging activity [2].

To 0.5 ml of various concentrations of extract, 0.3 ml of ABTS radical solution and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract, methanol was taken. The absorbance was measured at 734 nm. The experiment was performed in triplicate.

3. Reduction of ferric ions by O-Phenanthroline colour method [1].

The reaction mixture consisting of 1ml ortho-Phenanthroline, 2 ml ferric chloride (200 μM) & 2 ml of various concentrations of the extract were incubated at ambient temperature for 10 min. Then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate.

Experimental animals

Healthy adult male albino mice of Wistar strain weighing between 25-35g were used for the study. The mice were housed six in a cage, maintained in a temperature regulated and humidity controlled environment. The mice were fed with standard food pellets and water. Study was conducted after obtaining ethical committee

clearance from the Institutional Animal Ethics Committee of K.M.C., Manipal No. IAEC/KMC/06/2006-2007.

Acute Toxicity Studies [5]
Acute toxicity studies were conducted to determine the safe dose as per OECD guidelines. Three groups for the extract containing two animals in each group were used. Drugs were administered intraperitonially. After administration, the animals were observed continuously for one hour, frequently for the next four hours and then after 24 hours.

Experimental protocol:
Group I- Tumor control.
Group II- Cisplatin.
Group III- Alcoholic extract of *Clerodendron paniculatum* dose A (200 mg/kg)
Group IV- Alcoholic extract of *Clerodendron paniculatum* dose B (400 mg/kg)

a) **Selection of dose:**
The doses selected for the extracts were about $1/10^{th}$ of the safe dose found in acute toxicity studies. They were administered after 24 hours of tumor inoculation, once daily by intraperitonial route for 9 days.

Standard
The dose of Cisplatin selected was 3.5 mg/kg. This was calculated by using body mass index and past experience with the drug.

Liquid tumor model using EAC cell lines
The ascitic carcinoma bearing mice (donor) was taken 15 days after tumor transplantation. The ascitic fluid is drawn using a 22 gauge needle into sterile syringe. A small amount tested for microbial contamination. Tumor viability was determined by Trypan blue exclusion test and cells were counted using Haemocytometer. The Ascitic fluid was suitably diluted in phosphate buffer saline to get a concentration of 10^6 cells /ml of tumor cell suspension. This was injected intraperitonially to obtain ascitic tumor. The mice were weighed on the day of tumor inoculation and then for three subsequent days. Treatment was started 24 hours after tumor

inoculation. Cisplatin was injected on two alternate days i.e. the 1st and 3rd day. Extracts were administered till 9th day intraperitonially.

Parameters Monitored:

1. % increase in weight as compared to day- 0 weights. [3]
2. Mean survival time (MEST) and percentage increase in life span (% ILS).

1. % Increase in weight as compared to day "o"weight

Upon weighing the animals on the day of tumor inoculation and after once in 3 days in the post inoculation period the % increase in weight was calculated as follows

$$\left(\frac{\text{Animal wt on respective Day}}{\text{Animal wt on day 0}} - 1 \right) \times 100$$

2. Median survival time and increase in life span [%ILS]

Total number of days an animal survived from the day of tumor inoculation was counted. . Subsequently the mean survival time was calculated. The %ILS was calculated as follows.

$$\%\ ILS = \frac{\text{MST of treated group- MST of control group}}{\text{MST of control group}} X\ 100$$

An enhancement of life span by 25% or more over that of control was considered as effective antitumor response.

Solid tumor model using DLA cell lines

The DLA bearing mouse was taken 15 days after tumor transplantation. The ascitic fluid was drawn using a 22 guage needle into a sterile syringe. A small amount was tested for microbial

contamination. Tumor viability was determined by Trypan blue exclusion method and cells were counted using haemocytometer. The ascitic fluid was suitably diluted in phosphate buffer saline to get a concentration of 10^6 cells per ml of tumor cell suspension. Around 0.1ml of this solution was injected subcutaneously to the right hind limb of the mice to produce solid tumor. Treatment was started 24 hours after tumor inoculation. Cisplatin was injected on two alternate days i.e. the 1^{st} and 3^{rd} day. Extracts were administered till 9^{th} day intraperitonially.

Tumor volume [4]

The diameter of developing tumor were measured using a vernier calipers at three days interval for one month and tumor volume was calculated using the formula

$V=0.4\ ab^2$

Where a and b represents the major and minor diameters respectively.

Statistical analysis

The data obtained were analyzed by one way ANOVA using the software Origin. Level of Significance was fixed as $p<0.05$.

Results

Acute toxicity studies

Administration of alcoholic extract of *Clerodendron paniculatum* produced no observable side effects, upto 2000-mg/kg body weight in mice even after 72 hour of observation

Antioxidant studies

The alcoholic extract of *Clerodendron paniculatum* shows potent antioxidant activity. The concentration dependent scavenging of DPPH, ABTS, and O Phenanthroline were studied with the concentrations of 2µg/ml to 1024µg/ml

DPPH model

Table 1

Sl. No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	0.0	0.466		0.487	
2	2	0.453	2.77	0.440	9.56
3	4	0.444	4.78	0.349	28.24
4	8	0.435	6.71	0.233	52.08
5	16	0.412	11.67	0.049	89.76
6	32	0.384	17.56	0.032	93.27
7	64	0.332	28.78	0.020	95.73
8	128	0.291	37.57	0.019	95.92
9	256	0.195	58.25	0.020	95.81
10	512	0.096	79.37	0.062	87.17
11	1024	0.066	85.89	0.080	83.58

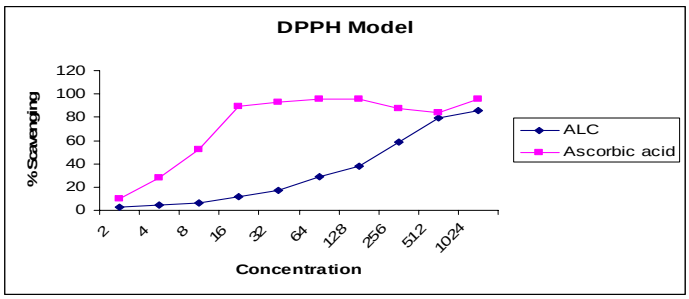

Fig.1: DPPH radical scavenging activity of different concentrations of *Clerodendron paniculatum* and ascorbic acid.

Reduction of ferric ions (O-phenanthroline method)
Table 2

Sl. No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	0.0	0.112		0.020	
2	2	0.118	5.08	0.058	64.48
3	4	0.122	8.19	0.061	66.34
4	8	0.126	11.11	0.073	72.05
5	16	0.157	28.66	0.090	77.19
6	32	0.174	35.63	0.107	80.74
7	64	0.207	45.89	0.132	84.50
8	128	0.253	55.73	0.150	86.12
9	256	0.295	62.03	0.175	88.24
10	512	0.427	73.77	0.200	89.70
11	1024	0.434	74.19	0.229	91.02

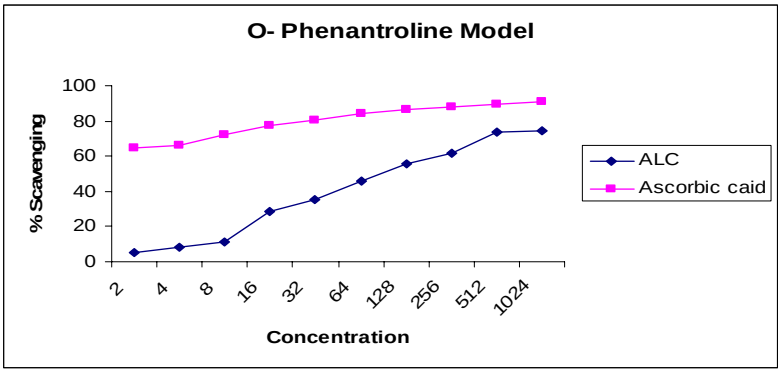

Fig.2 : O-phenanthroline radical scavenging activity of different concentrations of *Clerodendron paniculatum* and ascorbic acid.

ABTS radical scavenging
Table 3

Sl. No.	Conc. µg/ml	Alcoholic extract		Ascorbic acid	
		Abs	% Sca.	Abs	% Sca.
1	0.0	0.258		0.3637	
2	2	0.195	24.41	0.3403	6.43
3	4	0.165	36.04	0.3240	10.92
4	8	0.125	51.55	0.2316	36.32
5	16	0.099	61.62	0.1741	52.13
6	32	0.062	75.96	0.0050	98.63
7	64	0.030	88.37	0.0029	99.42
8	128	0.022	91.47	0.0020	99.45
9	256	0.044	82.94	0.0018	99.50
10	512	0.070	72.86	0.0012	99.67
11	1024	0.086	66.66	0.0015	99.58

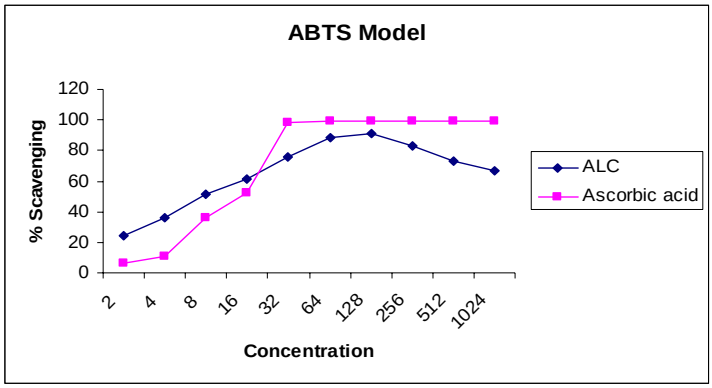

Fig 3: ABTS radical scavenging activity of different concentrations of *Clerodendron paniculatum* and ascorbic acid.

ANTICANCER STUDIES

Effect of alcoholic extract of *Clerodendron paniculatum* against EAC induced liquid tumor model in mice.

Table 4

Group	Dose mg/kg	Mean survival time Days	% ILS
CON	**-**	**16.33**	**-**
CIS	**3.5**	**32**	**97.48**
ALC	**200**	**18.75**	**14.81**
ALC	**400**	**19.33**	**18.37**

CON : Control
CIS : Cisplatin
ALC : Alcohol

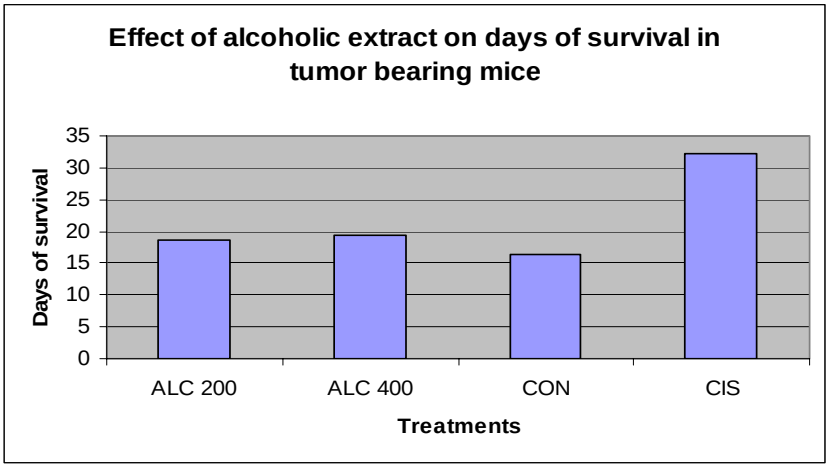

Fig.4: Effect of alcoholic extract on days of survival in tumor bearing mice

Effect of alcoholic extract of *Clerodendron paniculatum* against DLA induced solid tumor model in mice.

Table 5

Sl. No.	Groups (n=6)	Dose (mg/kg)	Volume of tumor on 4th week
1	CON	-	2.333
2	CIS	3.5	0.657
3	ALC	200	1.325
4	ALC	400	1.653

CON : Control
CIS : Cisplatin
ALC : Alcohol

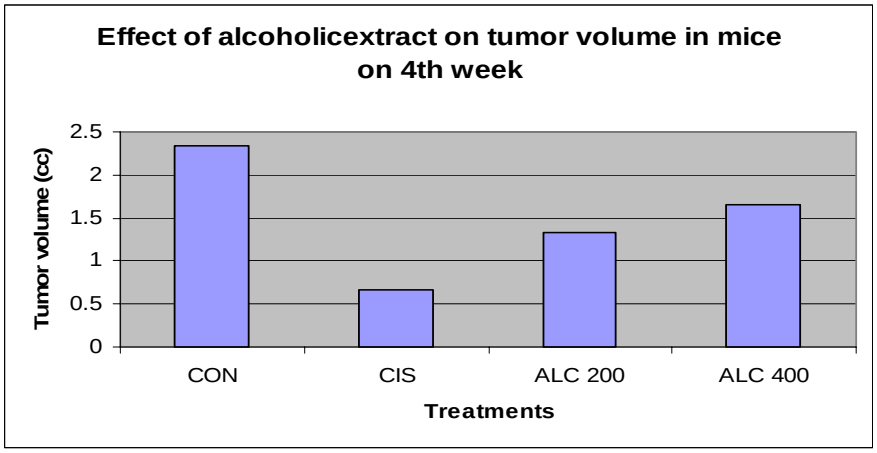

Fig. 5: Effect of alcoholic extract of *Clerodendron paniculatum* on tumor volume in mice on 4th week.

Discussion

The present study was undertaken to establish the free radical scavenging and anticancer activity of the *Clerodendron paniculatum*.

The alcoholic extract showed significant free radical scavenging effect on DPPH (table 1), ABTS (table 3) and Ferric ion free radicals (table 2). Alcoholic extract was tested for their *in vivo* anticancer potential by using liquid tumor model as well as solid tumor model. In the liquid tumor model, the alcoholic extract increased the survival time but it was not significant when compared to that of control (table4). The extract decreased the tumor volume in the solid tumor model but the reduction was not significant when compared to control (table5).

From the present study it is clear that *Clerodendron paniculatum* shows potent antioxidant activity. The study also shows that the plant possess anticancer potential but not significant. The plant may show activity at higher concentrations. Further studies should be carried out.

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