

Invitro and Invivo anticancer activity of Ethanolic extract of *Canthium Parviflorum Lam* on DLA and Hela cell lines.

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Abstract

Background: Wild Jessamine, *Canthium Parviflorum Lam*, (fam: Rubiaceae) is traditionally used for snake bite in some villages in shimoga district of Karnataka. *Canthium* Species are used in the treatment of tumor, cough, astringent and anthelmintic.

Objective: In this study, invitro and invivo anticancer activity of crude ethanolic extracts from the leaves of *Canthium Parviflorum Lam* was investigated

Method: The invitro anticancer activity was measured by MTT assay and Exclusion method. The invivo studies was determined in mice using Dalton's lymphoma ascetic (DLA) cells.

Results: The ethanolic extracts of *C.Parviflorum* greatly inhibited DLA and Hela cell growth with IC₅₀ Of 61.24µg/ml and 43.15µg/ml respectively. A significant increase in the life span and a decrease in the cancer cell number & tumour weight were noted in the tumor induced mice after treatment with *Canthium Parviflorum Lam*.

Conclusion: Anticancer activity of *Canthium Parviflorum* was may be due to flavonoid present in the plant. Further studies are also in process to evaluate the most potent fraction of the plant and to isolate the constituents of the fractions.

Key words:

Hela cell line, MTT assay, DLA cells, *Canthium Parviflorum*, Tumor

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1. INTRODUCTION

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host. The Chemotherapeutic agents are effective against various types of tumor are not totally free from side effects. This fostered our attempt to evaluate some plant products against cancer, as they are less likely to cause side effects. Many Indian species [1] and Plants[2] are quoted to be useful in different types of cancer. *Canthium parviflorum Lam* belonging to the family Rubiaceae. This plant is distributed in the Deccan peninsula from Gujarat and Maharashtra southwards, and in Bihar and Orissa[3]. *Canthium parviflorum Lam* has very limited systematically carried out investigations. The traditional systems of medicine use this plant alone or in combination with other medicinal plants for the treatment of various diseases. It was found from the tribes of south India that the plant has been used for the suppression of tumor like syndrome among their own population[4]. A vast literature collection fails to produce a scientific evidence to prove the anti tumor activity of *Canthium parviflorum Lam*. Hence this study was planned to evaluate Invitro and Invivo anticancer activity of ethanolic extract of leaves of *Canthium parviflorum Lam*.

2. MATERIALS AND METHODS

2.1. Plant Material

The fresh leaf of *Canthium Parviflorum* was obtained from Kanchipuram, Tamilnadu and the plant material was identified and authenticated by Prof Jayaraman, PARC, Chennai. Voucher specimen were deposited in the Pharmacognosy department, C.L.B.M College of Pharmacy, Chennai. The shade dried leaves was minced and extracted with 70% ethanol. The ethanolic extract of *C.Parviflorum* (EECP) was used to derive a series of concentration used in this paper. The Phytochemical screening proves the presence of carbohydrate, alkaloid and flavonoids[5].

2.2. Cell Culture[6]

Hela cell line was obtained from National centre for cell sciences, Pune, India. Stock cells are routinely cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 units/ ml pencillin and 100µg/ml streptomycin at 37° C an incubator containing 5% CO₂.

2.3. Experimental Animals [7]

Colonies inbred strains of swiss albino male mice weighing (20-25 gm),a obtained from C. L. Baid Metha College of pharmacy was used for the pharmacological studies. The animals were kept under standard conditions maintained at 23-25°C, 12 hr light/dark cycle and given standard pellet diet (Hindustan lever, Bangalore) provided *ad libitum*. The animals were acclimatized to the laboratory conditions for a week prior to the experimentation and randomly divided into six groups of each six animals. Principles of animal handling were strictly adhered to the guidelines and handling of animals was made under the supervision of animal ethics committee of the institute. The experimental protocol was approved by Institutional animal ethics committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

IAEC Reference number:
(IAEC/XXIX/10/2010).

2.4. Acute Toxicity studies [8]

This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD). A single administration of starting dose of 2000 mg/kg body weight/po of the EECP was administered to three female mice, and the mice were observed for three days to evaluate considerable changes in body weight and other signs of toxicity.

Repeating the experiment with the same dose level of EECP for more seven days, we observed the body

weight change and toxicity sign for totally fourteen days.

2.4. Invitro cytotoxic activity of EECP on DLA cells [9]

DLA cell lines was aspirated from the peritoneal cavity and washed 3 times with phosphate buffer saline solution. One million cells were incubated with various concentrations (12.5µg/ml, 25µg/ml, 50µg/ml & 100µg/ml) of the extract in a total volume of 1 ml for 3 hrs at 37° c. After incubation, the viability of the cells was determined by Trypan blue dye exclusion method

2.5. Invitro cytotoxic activity of EECP on Hela cells:

MTT ASSAY^[10]

MTT assay was performed as described (wang et al, 2001). Briefly cells were seeded at a concentration of 1.5×10^4 cells/ml in a 96 well plate. After overnight incubation, serial concentration of EECP (12.5µg/ml, 25µg/ml, 50µg/ml & 100µg/ml) were added. Since 1% ethanol was added to drug treatment group. Positive control (5-Flurouracil), Control group was added with 1% ethanol. Each concentration was repeated three times. These cells were incubated in a humidified atmosphere with 5% CO₂ for 3 days. Then 20µl MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was added to each well and incubated at 37° C for 4 hrs. The medium was removed and formazon was dissolved in DMSO and the absorbance is taken at 570nm. The growth inhibition was determined using Growth inhibition = (control O.D – sample O.D/ control O.D) and further IC₅₀ value were determined.

2.6. INVIVO ANTI-CANCER ACTIVITY

2.6.1. Effect of EECP on survival time [11]

Animals were inoculated with 1×10^6 cells per mouse on day 0 and treatment with EECP started 24 hours after inoculation at a dose of 200 mg/kg and 400 mg/kg/ day p.o. The control group was treated with

the same volume of 0.9% sodium chloride solution. All the treatments were continued for 10 days and observation was carried out for 45 days. The mean survival time (MST) of each group consisting of 6 mice, and changes in body weight was noted. The antitumour efficacy of EECP was compared with that of 5-fluorouracil (20mg/ kg, i.p) . The MST of the treated group was compared with that of the control group using the following formula. $MST = (T-C)/C \times 100$ T=Treated group C= Control group

2.6.2. Effect of EECP on haematological parameters:

In order to observe the effects of EECP on the haematological parameters of DLA bearing mice comparison was made amongst four groups (n = 6) of mice on the 14th day after transplantation. The four groups comprised of (1) tumour bearing mice (2) and (3) tumour bearing mice treated with EECP (200 mg/kg and 400 mg/kg bwt respectively) and (4) normal mice. Blood was drawn from each mouse from tail vein under sterilised condition and the Red Blood Cell Count (RBC) Haemoglobin content (Hb) and white blood cell count (WBC) [12] were studied using cell diluting fluids and a haemocytometer. Differential cell count was (DC) carried out from Leishman stained blood smears [13].

Statistical Analysis:

All values are expressed as mean \pm SEM. The data were statistically analysed by ONE WAY ANOVA followed by Tukey Kramer multiple comparison test. P values < 0.001 were considered as highly significant and <0.05 were considered significant.

3. Results and discussion

EECP were found to be cytotoxic towards DLA and Hela cells only at higher concentration. MTT assay is based on the reduction of MTT(3-(4,5- dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. . The IC₅₀ value of ethanolic extract showed

significant anticancer activity by exclusion method and MTT assay (Table 1, Figure 1). The mean survival time of tumour control group was 21 days. The EECP in both doses (200 & 400 mg/kg i.p) increased significantly the MST, 32 days in lower dose treated group ($P < 0.01$) and 41 days in higher dose treated group ($P < 0.001$). The results were almost comparable with the 5-fluorouracil treated group. (Table 2, Figure 2) EECP at both the doses (200 & 400 mg/kg p.o.) reversed the increased values in the haematological parameters induced by tumour to normal values. Tumour growth normally affects various haematological parameters and the anticancer activity is generally assessed by restoration of the changes in these parameters to normal and most significantly in decreased WBC and increased RBC, lymphocyte and haemoglobin content as compared to tumour control. The acceptance criteria for determining the antitumour activity of a compound is the determination of circulating WBC and the life span prolongation. [4] (Table - 3). Usually, in cancer chemotherapy, the major problems that are being encountered are of myelosuppression and anaemia the anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic condition. But the results have clearly shown that EECP (400mg/ kg) has not only brought back the bone marrow nucleated cell count to normal, but also the haemoglobin content and the RBC count to normal.

4. Conclusion

On the basis of the above results it can be concluded that the EECP possess significant anticancer activity studied by invitro and invivo models. The study also provides a strong evidence for the use of the leaves *Canthium parviflorum* in folklore treatment as anticancer agent. The activity may be due to the presence of one or more phytochemical constituents

present in the extract. Further studies warranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity.

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Table 1: The IC₅₀ values of EECP against Hela and DLA cell lines

Sample	Hela cell line (µg/ml)	DLA cell line (µg/ml)
EECP	43.15±1.6 *	61.24±1.7*
Standard (5-Fluorouracil)	20.30±2.1 *	26.34±2.2*

Values are expressed as mean ±SEM of 6 animals. The data were statistically analysed by ONE WAY ANOVA followed by Tukey Kramer multiple comparison test.

Table 2: Effect of EECP on the survival of tumour bearing mice

Treatment	Mean survival days	Increase in life span(%)
Tumor control	21±1.1	-
5-Fluorouracil(20mg/kg)	42±1.6*	89.4*
EECP (200mg/kg)	32±1.4*	60*
EECP (400mg/kg)	41±1.7*	88.6*

Values are expressed as mean ±SEM of 6 animals. The data were statistically analysed by ONE WAY ANOVA followed by Tukey Kramer multiple comparison test.

Table 3: Effect of EECP on haematological parameters

Treatment	Hb (g%)	RBC 10 ⁶ /mm ³	WBC/mm ³	Lymphocytes
Normal mice	11.5±4.9	10.86±0.96	5000±7000	74±3.52
Tumour bearing mice (Control)	3.45±0.61	5.2±0.76	43,850±1.86	58±5.58
EECP Treated tumor bearing mice (200mg/kg p.o)	9.2±0.36*	8.84±0.03*	17,533±1.33*	66±3.42*
EECP Treated tumor bearing mice (400mg/kg p.o)	10.6±0.40**	9.92±0.22**	16,583±1.96**	70±4.56**

Values are expressed as mean ±SEM of 6 animals. The data were statistically analysed by ONE WAY ANOVA followed by Tukey Kramer multiple comparison test.

Figure 1: The IC₅₀ values of EECP against Hela and DLA cell lines

The IC₅₀ values of EECP against Hela and DLA cell lines

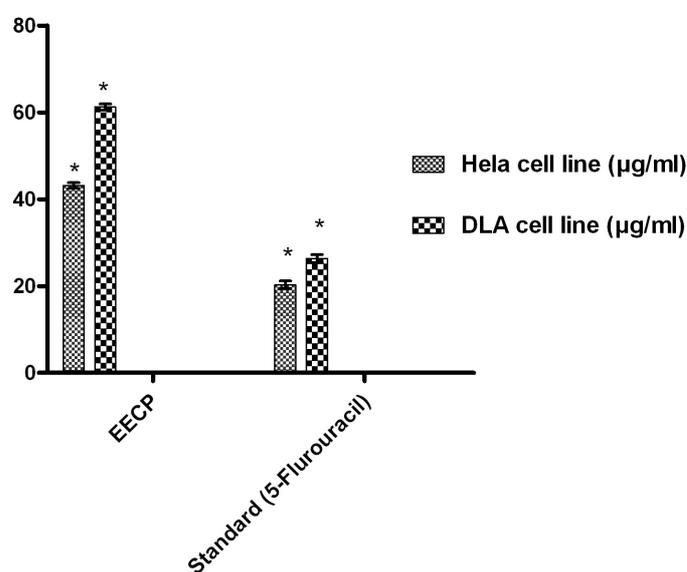
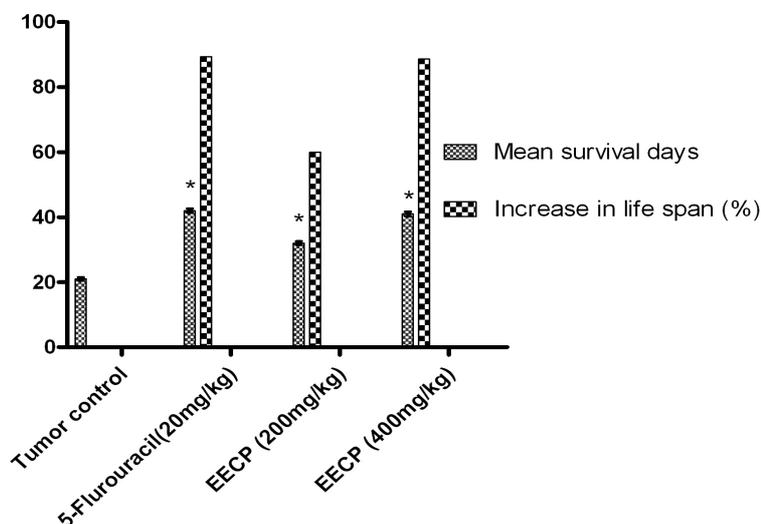


Figure 2: Effect of EECP on the survival of tumour bearing mice

Effect of EECP on the survival of tumour bearing mice



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