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Anticancer Activity of *Clerodendron infortunatum* Linn. Extract in Swiss Albino Mice

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The methanolic extract of *Clerodendron infortunatum* (MECI) was evaluated for anticancer activity against Ehrlich Ascites Carcinoma (EAC) in swiss albino mice. On day 1, the extract of leaves of *Clerodendron infortunatum* at a dose of 250 and 500 mg/kg body (b.w.) weight was administered intraperitoneal (i.p.) route and continued for 9 consecutive days. The anticancer activity of MECI was examined by determining the mean survival time, increase of life span, tumour volume, viable tumour cell count and non viable tumour cell count in experimental animal models. The extract increased the life span of Ehrlich Ascites Carcinoma treated mice and restored the haematological parameters as compared with the Ehrlich Ascites Carcinoma bearing mice. Thus, the present study suggests that the methanolic extract of *Clerodendron infortunatum* showed anticancer activity in tested animal models.

Key Words: *Clerodendron infortunatum*, Ehrlich Ascites Carcinoma (EAC), Anticancer.

INTRODUCTION

Cancer is one of the most common causes of carnage in all over the world. The aim of much research has been on the identification of natural and synthetic compounds that can be used in the prevention or treatment of cancer. Several methods exist for the treatment of cancer in modern medicine. These include chemotherapy, radiotherapy and surgery. Chemotherapy is now considered as the most effective method of cancer treatment. Intervention with chemo preventive agents at the early stage in carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumours with chemotherapeutic drugs. However, most cancer chemotherapeutic agents dramatically affect the host normal cells[1]. Some antitumor agents are also associated with toxic effects such as cardiomyopathy, nephrotoxicity and pulmonary fibrosis[2]. A major challenge for medical oncology is to develop therapeutic modalities that will prevent toxicity induced by antineoplastic treatments without impairing their antitumor effect. Hence, the use of natural products now has been contemplated of exceptional value in the control of cancer and its eradication program.

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Clerodendron infortunatum Linn. belonging to family Verbenaceae, have been used in Indian folk medicine in the treatment of bronchitis, asthma, fever, burning sensation, disease of blood, inflammation and epilepsy[3]. Traditionally, the plant is used as an antipyretic and antihelmentic. Leaves of the plant are prescribed for tumour, certain skin diseases and scorpion sting[4]. Previous phytochemical investigation of the plant revealed the presence of alkyl sterols[5] and 2,-(3,4-dehydroxyphenyl) ethanol 1-O-α-2 rhamnopyranosyl-(1 → 3)-β-D-(4-O-caffeoyl) glycopyranoside (acteoside)[6]. The aim of the present study is to evaluate the anticancer activity of the methanolic extract of leaves of *Clerodendron infortunatum* (MECI) against Ehrlich Ascites Carcinoma (EAC) in swiss albino mice.

EXPERIMENTAL

Plant material and preparation of extract: The plant *Clerodendron infortunatum* Linn. was collected in the month of November 2008 from the forest region of Midnapore, West Bengal, India. The taxonomical identification of the plant was done by M.S. Mondal, Botanical Survey of India, Shibpur, India and the voucher specimen (PMU-4/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference. The leaves of the *Clerodendron infortunatum* was dried under shade and then powered by mechanical grinder. The powder plant material was extracted with 80 % methanol using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (yield 12.5 % w/w). The extracts were stored in a vacuum dessicator for further use.

Animal used: Swiss albino mice weighing (20-25 g) were maintained in identical laboratory conditions (25-30 ºC temperature and relative humidity of 55-65 % with alternate light and darkness 12 h each) and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All procedures described were reviewed and approved by Jadavpur University animal ethical committee (ref No. 367001/C/CPCACA).

Acute toxicity: As per reported method[7], the LD_{50} value of MECI in swiss albino mice was determined.

Tumour cells: Ehrlich Ascites Carcinoma Cells were obtained from Chittaranjan National Cancer Institute (CNCI, Kolkata, India). The Ehrlich Ascites Carcinoma cells were maintained by weekly intraperitoneal inoculation of 2×10^6 cells/mouse.

Treatment schedule: Swiss albino mice were divided in to five groups of 12 animals (n = 12) each. The Ehrlich Ascites Carcinoma cells were collected from the donor mouse and were suspended in sterile isotonic saline (0.9 % w/v, NaCl). The Ehrlich Ascites Carcinoma cells were counted by treating with trypan blue solution as indicator under the microscope and it was confirmed as 95 % of viable cells. From this, 2×10^6 cells were injected intraperitoneally to each mouse. All the groups except first group received 0.1 mL Ehrlich Ascites Carcinoma cell suspension (2×10^6 cells/mouse, i.p.). This was taken as day '0'. The first group serve as normal

saline control. The second group served as Ehrlich Ascites Carcinoma control. After 24 h of tumour inoculation the third and fourth groups received MECI at a dose 250 and 500 mg/kg body weight, i.p., respectively and fifth group received reference drug 5-flurouracil (20 mg/kg b.w., i.p) for 9 consecutive days. 24 h after the last dose and 18 h of fasting blood was collected from six mice of each group by cardiac puncture for the estimation of hermatological parameters and then sacrificed by cervical dislocation for study of antitumor activity. The rest six mice of each group were kept with food and water ad libitum to check the increase in life span of the tumour hosts[8].

The effect of methanol extract on tumour growth and host's survival time were examined by studying the following parameters: mean survival time, increase in life span, tumour volume, tumour cell count, viable tumour cell count and nonviable tumour cell count[9].

Determination of tumour volume: The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min.

Determination of tumour cell count: The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in 64 small squares was counted.

Estimation of viable tumour cell count: The cells were then stained with Trypan blue (0.9 % in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and nonviable cells were counted.

$$\text{Cell count} = (\text{No. of cells} \times \text{dilution})/(\text{area} \times \text{thickness of liquid film})$$

Determinations of percentage increases life span and mean survival time: Recording the mortality monitored the effect of the methanol extract of *Clerodendron infortunatum* on tumour growth and percentage increase in life span (ILS %) were calculated[10].

$$\text{ILS (\%)} = [(\text{Mean survival of treated group/mean survival of control group}) - 1] \times 100$$

$$\text{Mean survival time} = [1\text{st death} + \text{last death}]/2$$

Hematological studies: The effect of the methanol extract of *Clerodendron infortunatum* on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin were done by standard procedures from freely flowing tail vein blood[11].

RESULTS AND DISCUSSION

The methanolic extract of *Clerodendron infortunatum* (MECI) at the dose of 250 and 500 mg\kg body weight increased the life span and non-viable cell count, decreased tumor volume and viable cell count of the tumor bearing mice, when

compared to that of Ehrlich Ascites Carcinoma control mice (Table-1). Methanolic extract of *Clerodendron infortunatum* also restored the hematological parameters. The number of RBC count and hemoglobin content also increased as compared to that of Ehrlich Ascites Carcinoma control. In the differential count the percentage of lymphocytes was increased with decreased level of neutrophils (Table-2).

TABLE-1
EFFECT OF METHANOLIC EXTRACT OF *Clerodendron infortunatum* ON MEAN SURVIVAL TIME (%) INCREASE OF LIFE SPAN, TUMOUR VOLUME, PERCENTAGE RATIO OF VIABLE AND NON VIABLE CELL IN EHRLICH ASCITES CARCINOMA BEARING MICE

Groups	Mean survival time (days)	Increase of life span (%)	Tumour volume (mL)	Viable cell count (%)	Non viable cell count (%)
Normal (0.9 % w/v NaCl)	–	–	–	–	–
EAC control (2×10^6 cells/mouse)	19.5	–	5.12 ± 0.36	78.82 ± 1.32	18.32 ±1.20
EAC + MECI (250 mg/kg)	31.0	58.97	2.42 ± 0.14*	38.46 ± 1.15*	56.64 ± 1.04*
EAC + MECI (500 mg/kg)	37.5	92.30	1.24 ± 0.22*	23.12 ± 1.43*	72.67 ± 1.26*
EAC + 5-F. uracil (20 mg/kg)	42.0	115.38	0.56 ± 0.05*	17.15 ± 0.32*	81.23 ± 1.06*

Statistical significance (p) calculated by one way ANOVA between the treated groups and the Ehrlich Ascites Carcinoma (EAC) control followed by Dunnett's test (*$p < 0.05$). MECI = Methanolic extract of *Clerodendron infortunatum*

TABLE-2
EFFECT OF METHANOL EXTRACT OF *Clerodendron infortunatum* EXTRACT ON HERMATOLOGICAL PARAMETERS IN EHRLICH ASCITES CARCINOMA BEARING MICE

Groups	Hb content (g %)	RBC (cell/ mL × 10^6)	WBC (cell/ mL × 10^3)	N	L	M
Normal (0.9 % w/v NaCl)	12.42±0.23	6.17±0.35	7.43±0.28	24.7±0.54	71.43±1.02	1.87±0.06
EAC control (2×10^6 cells/mouse)	6.3±0.34	3.80±0.12	11.72±0.33	68.73±0.73	28.25±1.21	1.02±0.04
EAC + MECI (250 mg/kg)	9.10±0.22*	4.95±0.43*	6.80±0.24*	36.62±0.46*	59.48±1.14*	1.50±0.5*
EAC + MECI (500 mg/kg)	11.20±0.46*	5.63±0.36*	7.10±0.48*	26.93±0.58*	68.42±1.32*	1.75±0.7*
EAC + 5-F.uracil (20 mg/kg	12.18±0.57*	5.85±0.38*	7.22±0.32*	25.35±0.69*	70.86±1.07*	1.79±0.8*

Statistical significance (p) calculated by one way ANOVA between the treated groups and the Ehrlich Ascites Carcinoma control followed by Dunnett's test (*$p < 0.05$). N: Neutrophils, L: Lymphocytes, M: Monocytes.

The present study shows that methanolic extract of *Clerodendron infortunatum* was significantly increased the life span than that of Ehrlich Ascites Carcinoma bearing mice. Moreover at the same time the standard drug 5-flurouracil also significantly increased the life span. It is documented that the reliable criteria for judging the value of any anticancer drug are prolongation of life span and decrease of WBC from blood[12]. The reduced volume of Ehrlich Ascites Carcinoma and increased survival time of mice suggest the delaying impact of methanolic extract of *Clerodendron infortunatum* on cell division. Generally in cancer chemotherapy, the major problem is anemia, due to reduction in RBC or hemoglobin concentration and leucocytes. Methanolic extract of *Clerodendron infortunatum* have significantly enhanced the erythrocyte count and hemoglobin level when compared to that of Ehrlich Ascites Carcinoma bearing mice. The WBC level is reduced when compared to that of Ehrlich Ascites Carcinoma bearing mice. These indicating parameters reveal that methanolic extract of *Clerodendron infortunatum* possess less toxic effect on hematological system. Viable cell count decreased with increased level of non-viable cell count. These suggested that methanolic extract of *Clerodendron infortunatum* have direct relationship with tumour cells. Because these tumour cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and these anticancer agents lysis the cells by direct cytotoxic mechanism.

Preliminary phytochemical examination showed that the presence of flavonoids, saponins and tannins in methanolic extract of *Clerodendron infortunatum*. Flavonoids have been shown to possess antimutagenic and antimalignant effects[13]. Further more, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation[14] and angiogenesis[15]. The cytotoxicity and anticancer properties are due to the presence of flavonoids.

Conclusion

The methanolic extract of *Clerodendron infortunatum* (MECI) increases life span and decrease tumour volume count in treated mice. Methanolic extract of *Clerodendron infortunatum* also possess less toxic effect on hematological system. So from the present study it is concluded that methanolic extract of *Clerodendron infortunatum* possess potent anticancer activity.

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REFERENCES

1. M. Mascarenhas, *Mushroom Res*., **3**, 77 (1994).
2. B. Nitha, C.R. Meera and K.K. Janardhanan, *Amala Res. Bull*., **25**, 165 (2005).
3. N. Sreevastava, *J. Med. Arom. Plant Sci. Biotech*., **1**, 142 (2007).
4. M. Yusuf, M.A. Wahab, J.U. Chowdhury and J. Begum,Medicinal plants of Bangladesh. Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh, p. 94 (1994).
5. T. Akihisa, Y. Matsubara, P. Ghosh, S. Thakur, T. Tamura and T. Matsumoto, *Steroids*, **53**, 625

(1989).
6. N.K. Sinha, K. Seth, V.B. Pandey, B. Dasgupta and A.H. Shah, *Planta Med*., **42**, 296 (1981).
7. J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
8. U.K. Mazumder, M. Gupta, S. Maity and M. Mukherjee, *Indian J. Exp. Biol*., **35**, 473 (1997).
9. M. Gupta, U.K. Mazumder, P.K. Haldar, C.C. Kandar, L. Manikandan and G.P. Senthil, *Iranian J. Pharm. Res*., **6**, 141 (2007).
10. P. Sur and D.K. Ganguly, *Planta Med*., **60**, 106 (1994).
11. J.V. Dacie and S.M. Lewis, Practical Hematology, J. and A Churchill, London, edn. 2, pp. 38-48 (1958).
12. C. Oberling and M. Guerin, *Adv. Cancer Res*., **2**, 353 (1954).
13. J.P. Brown, *Mutat Res*., **75**, 243 (1980).
14. G. Weber, F. Shen, N. Prajda, Y.A. Yeh and H. Yang, *Anticancer Res*., **16**, 3271 (1996).
15. T. Fotsis, M.S. Pepper, E. Akhtar, S. Breit, S. Rasku and H. Adlereutz, *Cancer Res*., **57**, 2916 (1997).

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