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Original Article

Antitumor activity of Leucopyrosinol isolated from the leaves parts of *Fluggea leucopyrus*

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ABSTRACT

The objective of the present study was to evaluate the anti-tumor activity of Leucopyrosinol isolated from the leaves parts of *Fluggea leucopyrus*. Antitumor activity of *Fluggea leucopyrus* against EAC was determined using single dose and intermittent treatments over two weeks, i.e. four-drug administration by the intrapretonial route on days, 1, 5, 9, and 11 after EAC cells inoculation. The compound and the vehicle were administered I.P using 10 mice per test group. Results indicated that there was significant increase in life span and reduction in the tumor volume in *Fluggea leucopyrus* treated mice. The results of the study strongly demonstrate that *Fluggea leucopyrus* exhibited significant antitumor activity against EAC in mice.

Keywords: *Fluggea leucopyrus*, Anti-tumor

INTRODUCTION

Cancer is the second leading cause of death worldwide.[1] Cancer chemoprevention is a relatively new concept. The pioneering work to reduce cancer incidence by chemical intervention was initiated by the groups of Wattenberg and Sporn in the early 1960s and 1970s.[2,3] Later, scientists have embraced the concept of cancer chemo prevention is a distinct new discipline of oncology .[4,5,6,7,8]

There is compelling evidence from epidemiological and experimental studies that highlight the importance of compounds derived from plants "phytochemicals" to reduce the risk of colon cancer and inhibit the development and spread of tumors in experimental animals. More than 25% of drugs used during the last 20 years are directly derived from plants, while the other 25% are chemically altered natural products. An ideal phytochemical is one that possesses anti-tumor properties with minimal toxicity and has a defined mechanism of action. As compounds that target specific signaling pathways are identified, researchers can envisage novel therapeutic approaches as well as a better understanding of the pathways involved in disease .[1] Over 60% of currently available anticancer agents are derived in one way or another from natural sources, including plants, marine organisms and microorganisms .[9,10,11]The search for anticancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic phodophyllotoxins. As a result, United States National Cancer Institute (NCI) initiated an extensive plant collection program in 1960, focused mainly in temperate regions. This lead to the discovery of many novel chemo types showing a range of cytotoxic activities.[12] including the taxanes and camptothecins, but their development into clinically active agents spanned a period of some 30 years, from the early 1960s to the 1990s. It is interesting to note, however that no new plant-derived clinical anti-1 agents have, as yet, reached the stage of general use, but a number of agents are in preclinical development [10] Oxidative stresses are related with various diseases and pathological conditions such as aging, atherosclerosis and cancer .[13,14,15]Antioxidants may prevent these chronic diseases by several mechanisms such as enzymatic degradation of free radicals, chelation of metals which stimulate the production of free radicals and scavenging the free radicals .[16]Medicinal plants are considered to be the important source of antioxidant compounds, and recently there has been considerable interest in finding the natural antioxidants from plant materials to replace synthetic ones .[17] Phototherapeutic products are many times mistakenly regarded as safe because they are natural .[18]Nevertheless, those products contain bioactive principles with potential to cause adverse

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effects [19]In addition poor pharmacovigilance services of this area make it difficult to determine the frequency of adverse effects caused by the use of phototherapeutic products.[20]Thus all the natural products used in the therapeutics must be submitted to efficiency and safety tests by the same methods used for new synthetic drugs .[21,22]

In the present study, we report for the first time the, anti-tumor, activity of methanol extract of *Fluggealeucopyrus*.

MATERIALS AND METHOD

Collection of plant materials: The leaves of Fluggea leucopyrus was collected from Thirupati forest region Thirupati district Andhra Pradesh. This plant species were authenticated by Prof. Madhava Chetty Botanist Department of Pharmacognosy and Photochemistry (Padmavathi Mahila Kalasala, Tirupati) .The collected plant material was washed thoroughly with water to remove the adhering soil, mud, and debris. The leaves were dried in shade at room temperature to a constant mass. The plant material was coarsely powdered into coarse powder a warring. The powder was stored in an airtight container and protect from light.

Preparation of extract: 1oogm powdered leaves parts were subjected to successive extraction in an extractor using methyl alcohol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get constant weight.

Mice and tumor model: Swiss albino mice of either sex (8-10 weeks old) weighing 20-25 g, were used for the experiment. The animals were maintained under proper environmental conditions i.e., temperature 25 ± 2ºC and humidity 50 ± 5% with a 12 h light and dark period. They were housed in polypropylene shoebox type cages with stainless steel grill top, bedded with rice husk. The animals were provided with pelleted diet (Gold Mohur, Lipton, India) and water *ad libitum*. 10 animals were used in each control and treated group. Swiss albino mice of either sex were used for implanting *Erlichascitis* tumor model.

Experimental Design: Mice (6-8 week old) were challenged with Ehrlich Ascetic carcinoma (EAC) cells (10^6cells/mouse; subcutaneously) on day zero. The treatment began the day after tumor inoculation, and test compound (***Fluggea leucopyrus***) and vehicle were administered ip using ten mice per test group. Routinely, the compound was administered four times: on the day after tumor inoculation (D1), on day D5, D9 and D11 (Scheme D1, 5, 9 and 11). The compound was also administered once (D1, single dose). In all chemotherapy trials mice were checked daily, with any adverse clinical reactions noted and deaths recorded. Mice were weighed 2-4 times weekly during treatment and once weekly thereafter. Tumors were measured by calipers twice weekly and tumor volume (mm^3) were estimated as = 0.5 (Length X $Width^2$). Results are presented for experiments involving ten mice per experimental group.

Evaluation of anti-tumor activity:

Life Span: Mortality was noted every day and the median life span was calculated. The mouse (Mm) separates into two identical groups (one group, including the mice that died before Mm, the other group including those who died after) and the median day (Dm) is the day Mm died. Mice surviving for at least 45 days were considered as cured and were included in the calculation of the median life span. Compound efficiency was expressed by T/C as follows:

T/C% = (MLS of treated animals/ MLS of control animals) X 100

Or by the increase in life span ILS: ILS% = 100 X (T-C) / C

The therapeutic index (defined as the ratio of the dose that kills 10% of tumor-free mice to the dose that gives a 50% increase in life span in tumor-bearing mice) was determined for each experiment. Survival curves of treated and control groups were statistically compared using the Log-rank test.

Tumor Growth: Treatment efficiency is assessed in terms of the compound's effects on the tumor volumes of tumor bearing mice relative to the control vehicle-treated mice. Two evaluation criteria were used in parallel: (i) Specific tumor growth delay (SGD), calculated as follows: for EAC tumor model = [Td (drug-treated group)-Td (vehicle treated group)]/ Td (vehicle-treated group), with Td being the tumor doubling time of drug - treated and control groups, defined as the time in days required for the tumor volume to double. (ii) Tumor regressions defined as partial (PR) if the tumor volume decreased to 50% or less of that at the start of treatment, without dropping below measurable size. [23]

RESULTS

Antitumor activity against EAC was determined for *Fluggea leucopyrus* using single dose and intermittent treatments over two weeks, i.e., four drug administration by the i.p., route on days, 1, 5, 9, and 11 after EAC cells inoculation. The compound and the vehicle were administered ip using 10 mice per test group. *Fluggea leucopyrus* exhibited antitumor activity against EAC, with a significant increase in life span. The effect of

Fluggea leucopyrus on the survival of tumor bearing mice is shown in the Table 1. There was a dose-effect relationship and the increase in life span. The ILS% was 14.18, 22.00 and 60.15 % for the single dose and 3.40, 33.75 and 71.00 % for the multiple doses at the dosage of 100, 150 and 200 mg/kg body weight respectively. There was reduction in the tumor volume of mice treated with *Fluggea leucopyrus*. The tumor volume of control animal on the 35th day of tumor injection was 2.0 ± 0.59ml, whereas the compound treated group it was 1.75 ± 0.47, 1.74 ±0.82, and 0.49 ± 0.98 ml for the single dose and 1.25 ± 0.60, 0.75 ± 0.43, and 0.25 ± 0.83 ml for the multiple dose respectively for the doses of *Fluggea leucopyrus* mentioned earlier. Seven or eight out of ten mice were cured with these compounds. The highest therapeutic index was obtained with multiple schedules. The results obtained for these compounds are shown in Figure 3a-b. Present compound *Fluggea leucopyrus* was shown to be non-toxic even when the concentration was increased up to 900 mg/kg body weight.

Table.1: Antitumor activity of *Fluggea leucopyrus* given ip against the sc implanted EAC.

Survival	Treatment Schedule	MTD [a] (mg/kg)	TI [b]	Dose (mg/kg)	SGD	ILS%
	D1	300	5.70	100	1.10	14.18	9/10
150	0.80	21.00		8/10		
200	0.72	60.15		6/10		
D1,5,9,11	300	5.72	100	< 4	3. 40	6/10
				150	0.60	33.75	6/10
				200	1.00	71.00	7/10

Tumour cells are inoculated ip as described in Materials and Methods; Drugs are then injected ip with in different schedules, and ILS% are then determined. [a] MTD – maximum tolerance dose; [b] TI, therapeutic index.

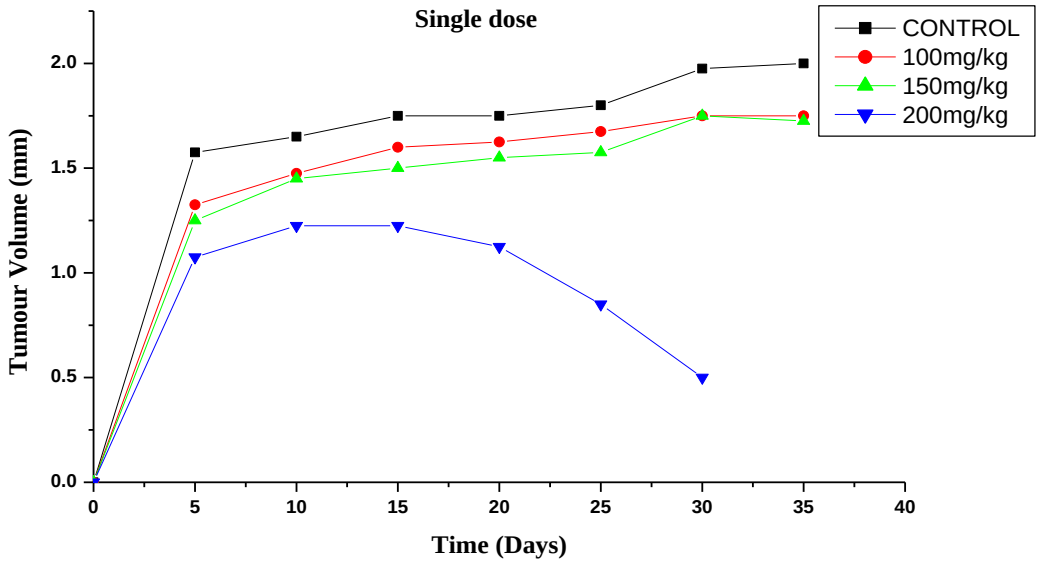

Figure.1: In vivo antitumour activity of leucopyrosinol on Ehrlichs Ascitic carcinoma. Tumors were generated by s.c., inoculation of EAC cells in Swiss albino mice. The compound was administered i.p., once (D1, Single dose) on the day after tumor inoculation. Average tumour volumes of treated (n = 10) and control (n = 10) are shown.

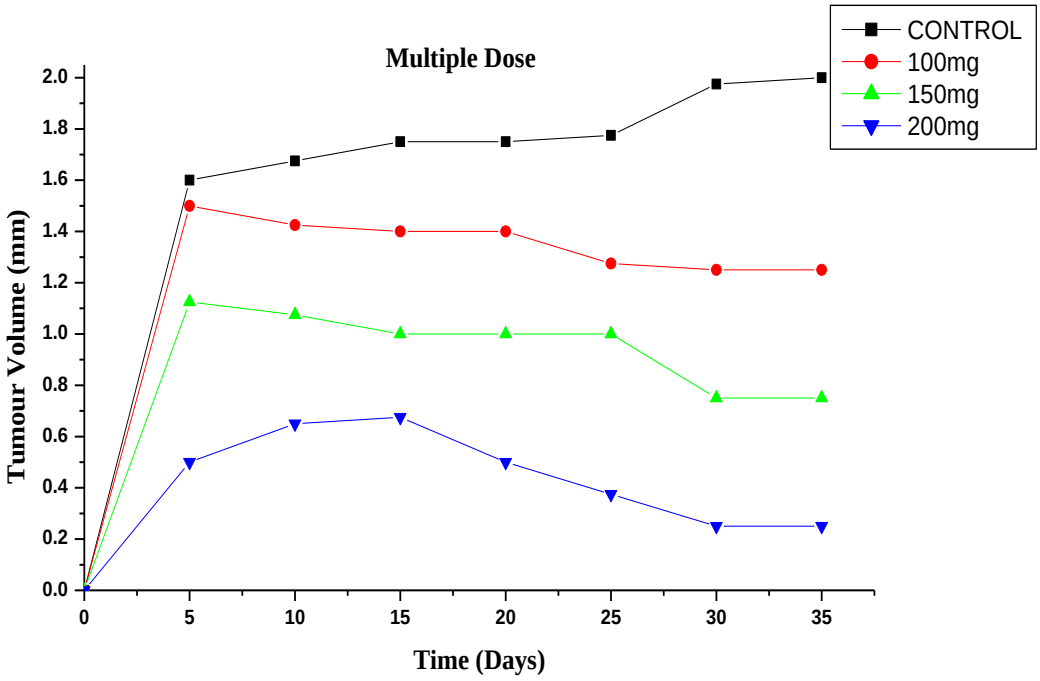

Figure.2: In vivo antitumour activity of leucopyrosinol on Ehrlichs Ascitic Carcinoma. Tumors were generated by s.c., inoculation of EAC cells in Swiss albino mice.) The compound was administered i.p., 4 times: on day D1, D5, D9 and D11 (Multiple doses). Average tumour volumes of treated (n = 10) and control (n = 10) are shown.

DISCUSSION

Anticancer drugs have well known therapeutic limitations and this has stimulated the search for new agents with enhanced therapeutic efficacy. Considerable efforts have been directed towards medicinal plants, which have been reported to be effective in the treatment of human cancers. Therefore, search for new drugs is required for the treatment of cancers.[24]Although the *Fluggea leucopyrus* has demonstrated significant in vivo antineoplastic activities against the tumour model.

The cytotoxic effects of plant flavonoids are shown to be mediated through apoptosis. Considering the ability of these natural flavonoids to absorb proteins and metal ions, there is a possibility that they can elicit apoptosis signals through various receptors or proteins. Apart from this, they are excellent antioxidants and they thus prevent free radical attack on DNA by acting as scavengers of these free radicals. A number of flavonoids are topo-II poisons inhibiting topo I/II isomerases thus enhancing the DNA cleavage.

Another possible mechanism of reported for anticancer drugs is inhibition of DNA synthesis and thus prevention of cell division. Folic acid supplied from the diet is essential for the production of tetrahydrofolic acid (THF). The conversion of folic acid to THF is carried out by an enzyme folate reductse. Anticancer drugs compete with folic acid for this enzyme thus restricting the production of THF required for synthesis of DNA and consequently for cell replication. Cells, which do not have adequate production of THF eventually, die.

It should be noted that there are a number of reports that the anticancer plant extracts retarded development of ascetic tumour growth and increased the life span of tumour growth and increased the ILS% .[24,25,26,27,28]For example, Loranthus extract significantly inhibited Ehrlichs Ascetic Carcinoma (EAC) growth in mice .[29]Preparations from Solanum trilobatum also reduced the growth of EAC in mice .[30]In the present study the methanol extract significantly increased the life span of ascetic tumour bearing mice dose dependently. Moreover, the extracts significantly reduced the solid tumor development in the mice.

CONCLUSION

In summary, this study shows the antitumor potential of *Fluggea leucopyrus* isolated from the leaves parts of *Fluggealeucopyrus*. showed a

significant activity against the in vivo tumor models. The high activity of *Fluggea leucopyrus* against EAC must be considered as a new class of anti-tumor agents**.**

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REFERENCES

1. Amin A, Fali-Muhtasib H, Ocker M, Schneider –Stock R. Overview of major classes of plant derived anticancer drugs. Int J Biomed Sci2009; 5:1-11.
2. Wattenbrg LW. Chemo prevention of cancer. Can Res1985; 45:1-8.
3. Sporn MB. Chemopreventiontion of cancer. Lancet1993;342: 1211-1213
4. Keloff GJ, Boone CW, Crowell JA, Steele VE, Lubet RA, and Sigman CC. Chemo preventive drug development: perspective and progress. Cancer Epdemiol Biomarkers prev1994; 3: 85-98.
5. Greenwald P, Kelloff G, Burch-Whitman C, and Kramer BS. Chemoprevention. CA Cancer J Clin1995; 45: 31-49
6. Hong WK, Sporn MB. Recent advances in chemo prevention of cancer. Science 1997; 278: 1073-1077.
7. Stoner GD, Morse MA, and Kelloff GJ Perspectives in cancer chemoprevention. Environ health persp1997; 105(suppl.): 945-954.
8. Trignali C. Bioactive compounds from natural sources; Isolation, charecterization and biological properties. Boca Raton, FL. 2001; 70-84.
9. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003; 66: 1022-1037.
10. Cragg GM, Newman, DJ. Plants as a source of anticancer agents. J Ethnopharmacol2005; 100: 72-79.
11. Cragg GM, Kingston DGI, Newman DJ. (Eds.). Anticancer agents from natural products. Brunner-Routledge Psychology Press, Taylor and Francis Group,Boca Raton, FL. 2005; 431-439.
12. Cassady JM, Douros JD (Eds). Anticancer agents Based on Natural Product Models.Academic press, New York.1980; 134-142.
13. Cadenas E, Davies KJ. Mitochondrial free radical generation oxidative stress and ageing. Free rad biol and Med2000; 29: 222-230
14. Aviram M. Reviews of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free rad res2000; 33: S85-S87.
15. Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, Takashi Y, Fukuzawa K. Novel antioxidants isolated from plants of the genera Ferula, Inula, Prangos and Rheum collected in Uzbekistan. Phytomed 2004; 11: 645-651.
16. Penckofer S, Schwertz D, FlerczakK . Oxidative stress and cardiovascular disease in type 2 diabetes: The role of antioxidants and pro-oxidants. J Cardiovascular nurs2002; 16: 68-85.
17. Mehdipour S, Yasa N, Dehghan G, Khorasani, Mohammadirad A, Rahimi, R, and Abdollahi M. Antioxidant potential of Iranian Carica papaya juice in vitro and in vivo are comparable to α-Tocopherol. Phytother Res 2006; 20: 591-594.
18. Gester MW. Therapeutic landscape: Medical issues in light of the new cultural geography. Social Sci and Med 1992; 34: 735-746.
19. Bent S, Ko R. Commonly used herbal medicines in the United States: a review. The American J Med 2004; 116: 478-485.
20. Eisnberg DM, Davis RB, Ettener SL. Trends in alternative medicine use in the United Sates, 1990-1997: results of a follow-up national survey. The J American Med Assoc 1998; 280: 1569-1575
21. Talay P, Talay P. The importance of using scientific principles in the development of medicinal agents from plants. Acad med2001; 76: 238-247.
22. Fères CAO, Madalosso RC, Rocha OA, Liete JPV, Guimarães TMDP, Toledo VPP, Tagliati CA. Acute and chronic toxicological studies of Dimorphandramollis in experimental animals. J Ethnopharmacol2006; 108:450-456.
23. Plowmann J, Dykes DJ, Hollingshied L, Simpson-Herren MC, Allaey. In: Anticancer drug development Guide, B.A. Teicher, Humana Press. Inc. Totowa NJ. 1997; 101-125.
24. Xingming M, Hongjuan Y, Ying D, Yanping L, Weihua T, Fangyu A, Jun G. Antitumor effects of ethanolic extracts from Sophoramoorcroftiana seeds in mice. Iranian Red Crescent MedlJl2009; 11: 18-22.
25. Abdel–Khader, Mahmoud AH, Motawa HM, Wabha HE, Ebrahim AY. Anti-tumor activity of Urticapilulifera on Ehrlich Ascites carcinoma in Mice. Asian J Biochem2007; 2(6): 375-385.
26. Rajkapoor B, Jayakar B, Murugesh N, Sakthisekaran . Chemoprevention and cytotoxic effect of Bauhinia variegata against N-nitrosodiethylamine induced liver tumors and human cancer cell lines. J Ethnopharmacol2004;104: 407-409.
27. Babu BH, Shylesh BS, Padikkala J. Tumor reducing and anti-carcinogenic activity of Acanthus ilcifolius in mice. J Ethnopharmacol2002; 79: 27-33.
28. Latha PG, Panikkar KR. Anti-tumor principals of Ixoracoccinea flowers. Cancer Lett1998; 130: 197-202.
29. Mary KT, Girija K, KuttanR.Partial purification of tumor reducing principle from Helianthus elasticus. Cancer Lett1994; 81:53-57.
30. Mohanan PV, Devi, KS. Cytotoxic potential of the preparation from Solanum tribolatum and the effect of sobatum on tumor reduction in mice. Cancer Letters1996; 110: 71-76.