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Evaluation In-Vitro and In-Vivo Anticancer Activity of Leaf Extarcts of Vitex Negundo by Different Cancer Cell Lines

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ABSTRACT:

Objective: Present study carried out to evaluate anticancer activity of leaf methanol and aqueous extracts of vitex negundo belongs to the family verbenaceae on the defferentcancerous and normal cell lines has an A549 cells(human lung),DLA tumour cells (Daltons lymphoma ascites) and vero normal cels(A.G monkey kidney).

Methods: Prepared leaf extracts were used as in vitro cytotoxicity against cell lines DLA tumour cellline and particular methanol extract shows significant result.So methanolic extract was taken futher invitro anti tumour activity by assessing biochemical parameters such as tumour volume ,viable and non viable tumour cell count, tomour weight and haematological estimations.

Results: cytotoxicity in-vitro cytotoxicity study, Me extract showed direct cytotoxic effect on the A-549 and DLA cell line in a concentration dependent manner andestimated the IC50 value was found to be 209.44 ± 2.07 µg/mL (Me) and 302.3 µg/mL (Aq) 173.37 µg/mL (Me) and 283.1 µg/mL (Aq) respectively while both the extract were less toxic to Vero cell line and IC50 value was found to be 164.05 µg/mL (Me) and 292.06 µg/mL (Aq) (Tbl.1) In vitro cytotoxicity study shows that Methanol extract is more cytotoxic for DLA tumour cells hence MeVC was taken for further in vivo anti tumour study. At doses of 200mg/kg and 400mg/kg MeVC exhibited significant ($P<0.01$) decrease in the tumor volume, viable cell count, tumor weight, and elevated the span of DLA tumor bearing mice. The hematological parameters were reverted to normal level in MeVC treated mice.

Conclusion: The present study states that the alcoholic leaf extract of Vitex negundo showed a significant in vitro and in vivo anti tumour activity against DLA cells and also less toxic for human cells.

Keywords: Anti cancer, A-549 cell line, Vero cell line, Cytotoxicity activity, Anti tumour, vitex negundo.

I. INTRODUCTION

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.1 Cancer is a large group of diseases, all of which have one thing in common i.e. cells growing out of control or fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered 2. Though many diseases (such as heart failure) may have a worst prognosis than most cases of cancer, cancer is the subject of widespread fear and taboos, there are 200 different types of cancer that afflict humans3. The causes of cancer are diverse, complex, and only partially understood. Many things are known to increase the risk of cancer, including tobacco use, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity, and environmental pollutants4. Cancers are classified by the type of cell that the tumor cells resemble and

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are therefore presumed to be the origin of the tumor. Although, several classes of anti cancer are currently being used, due to clinical limitations and adverse effects there is critical interest in development of efficient and safe drugs for treatment of cancer. vitex negundo Family — Verbenaceae leaf smoke is inhaled to get rid of cough14; in case of diarrhoea flowers are used69; extract of the plant is taken as a diuretic14. 'Muscle & joint rub'11, is a highly effective medicine for backache, muscular sprain and joint pain. 'Dental Cream'11 is a formulated toothpaste that tightens and reduces swelling of gums, stops gum bleeding, prevents toothache, decay and controls bad breath. 'Atharva Nirgundi Siddha Tail'134 is useful in arthritis, joint pain, relieves oedema. Thirty-five patents were found on its medicinal applications mainly for rheumatic arthritis 5. Present work aims to evaluate anti-cancer potency of vitex negundo leaves using various cancer and normal cell lines in vitro and in vivo.

II. MATERIALS AND METHODS

Collection of leafs and extraction

The fresh leaves of vitex negundo were collected in November 2019, from forest area of adilabad, telanagan, India. The plant was identified authenticated by the Dr. K.Raju , professor, Department of Botany; Kakatiya university warangal collection number1028 . For further confirmation, the microscopic characters of this plant was studied and compared with available literature as mentioned above. The collected whole plant (12kg) was air dried and powder. The powder was extracted by maceration with methanol at room temperature for 14days with occasional shaking. The methanolic & Aq. extract was filtered and concentrated under vacuum using rotary evaporator at low temperature (45°C).

Animals

Swiss albino mice of about eight weeks old were used for the experiment. The mice were grouped and housed in poly acrylic cages (38cm×23cm×10cm) with not more than six animals per cage. The animals were maintained under standard laboratory conditions (temperature 25±2°C and 55±5% relative humidity with dark/light cycle 14/10h) and were allowed free access to standard dry pellet diet and water ad libitum. The mice were acclimatized to laboratory condition for seven days before the commencement of the experiments.

Transplantation of tumor cells

DLA (Dalton's lymphoma Ascites) cells were procured from Amla Cancer Institute Amla nagar, Trissur, Kerala India. The cells were maintained in vivo in Swiss albino mice i.p. The asicitic fluid was drawn out from DLA tumor bearing mouse at log phase (days 7-8 of tumor bearing) of tumor cells. The cells withdrawn were used for both in vitro and in vivo study 6.

Assay for in vitro cytotoxicity study

In vitro cytotoxicity assay of Me and Aq was performed by using Vero (African green Monkey Kidney), A-549 (Human lung) and Dalton's Lymphoma Ascites (Tumour cells) cell line. Briefly, 1×106 cells were suspended in 0.1 ml of phosphate buffered saline (PBS, 0.2 M, pH 7.4) and mixed with 100µl of various concentration (25,50,100,150,200 and 300µg/ml) of MeVC and Aq.VC and standard drug 5-flurouracil. The final volume was adjusted 1 ml with PBS and was incubated at 37°C for 3 h after the incubation was over, the viability of the cells was determined using trypan blue (0.4% in normal saline) method and the percentage of cytotoxicity was determined by calculating percentage inhibition and IC50 value 7-8.

Acute toxicity

The acute oral toxicity of MeBG in Swiss albino mice was performed as per OECD guidelines . The extract was safe up to the dose of 2g/kg b.w. P.O. for mice. 9

Treatment schedule for assessment of in vivo antitumor potential

The Swiss albino mice (20-25 g) were divided into five groups (n=12). Except, Group-I all the animals in DLA group were being injected with DLA cells (2×106 cells/mouse, i.p). This was marked as day "0". Group-I was served as normal saline control (5 mL /kg, i.p.) and group-II was served as DLA control. After 24h, DLA transplanted group-III and IV were being injected MeVC (200 and 400mg/kg b.w. i.p.) once daily for 11 consecutive days. Group V received standard drug 5-Flurouracil (20 mg/kg i.p) for 11 consecutive days 10. After administrations of last dose 6 mice from each group were kept fasting for 18h and blood was collected by direct cardiac puncture for the estimation of haematological determination. Rest of animals in each groups were kept alive with food and water ad libitum to check the percentage increase in life span of the tumor

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host and also to determine the mean survival time (MST).Antitumor activity of Methanolic extract was assessed by observation of changes with respect to the following parameters .6

Tumor volume and weight

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. Volume of the fluid was measured by taking it in graduated centrifuge tube and expressed in millilitre (ml). Tumor weight was measured by taking the weight of the mice before and after the collection of the ascitic fluid from peritoneal cavity and expressed in gram (g).

Percentage increase life span (ILS)

The effect of MeVC on tumor growth was monitored by recording the mortality of the experimental mice. The percentage increase in life span (ILS) was calculated by the following formula: Mean survival time (MST) in days = (day of first death + day of last death)/2 ILS (%) = [(MST of the treated group/MST of the control group)-1] ×100

Tumor cell (Viable/nonviable) count

The ascitic fluid was taken in a pipette and diluted upto 20 times with PBS solution. Then a drop of the diluted cell suspension was being injected MeVC (200 and 400mg/kg b.w. i.p.) once daily for 11 consecutive days. Group V received standard drug 5-Flurouracil (20 mg/kg i.p) for 11 consecutive days10. After administrations of last dose 6 mice from each group were kept fasting for 18h and blood was collected by direct cardiac puncture for the estimation of haematological determination. Rest of animals in each groups were kept alive with food and water ad libitum to check the percentage increase in life span of the tumor host and also to determine the mean survival time (MST).Antitumor activity of MeVC extract was assessed by observation of changes with respect to the following parameters6 .

Tumor volume and weight

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. Volume of the fluid was measured by taking it in graduated centrifuge tube and expressed in millilitre (ml). Tumor weight was measured by taking the weight of the mice before and after the collection of the ascitic fluid from peritoneal cavity and expressed in gram (g).

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Tumor cell (Viable/nonviable) count

The ascitic fluid was taken in a pipette and diluted upto 20 times with PBS solution. Then a drop of the diluted cell suspension was placed on Neubauer's counting chamber and the number of cells in the 64 small squares were counted. The viability and non-viability of the cell were determined by tryphan blue assay. The cells were stained with tryphan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and non viable cells were counted using the under-mentioned formula: Cell count = (number of cells × dilution factor)/ (area × thickness of liquid film).

Haematological parameters

The collected blood was used for the estimation of hemoglobin (Hb), red blood cell (RBC) and white (WBC) count by standard procedures11.

Statistic analysis All the experimental data are expressed as the mean SEM. The data was statistically analyzed by using one way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by Instat using Graph Pad Prism 5.0.

III. RESULTS AND DISCUSSION

In in vitro cytotoxicity study of MeVC extract and Aq. extract, the Methanolic extract show direct cytotoxic effect on the A-549 and DLA cell line in a concentration dependent manner and the IC50 value was found to be 143.4 µg/ml (MeVC) and 210.8 µg/ml (AqVC) 137.2 µg/ml (MeVC) and 217.8 µg/ml (AqVC) respectively while both the extract were less toxic to Vero cell line and IC50 value was found to be 148.7 µg/ml (MeVC) and 152.6 µg/ml (AqVC) Table1.

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IC50 value by Trypan blue dye exclusion technique MeVC showed potent cytotoxicity activity against DLA and A- 549 cell line. (µg/mL).

Cells	Methanol	Aqueous
A-549	209.44 ± 2.07	302.3 ± 0.13
DLA cell line	173.37 ± 0.24	283.1 ± 0.11
Vero cell line	164.05 ± 0.77	292.06 ± 0.06
Methotrexata	0.3 **µg**	

The values presented are mean ± standard deviation, n = 3. Results were analyzed using descriptive statistics.

In vitro cytotoxicity study shows that MeVC is more cytotoxic for DLA tumour cells and less cytotoxic for normal cell line i.e. A-549 cell line hence MeVC was taken for further in vivo anti tumour study.Anti tumor activity of MeVC against DLA tumor bearing mice was assessed by the parameters such as tumor volume, tumor weight, cell count (viable and nonviable), mean survival time and percentage increase in life span. The tumor volume, tumor weight and viable cell count were found to be significantly (P<0.01) increased and non viable cell count was significantly (P<0.01) declined in DLA control animals, when compared with normal control animals (Table 2).

Table 2: Effect of MeBG on tumor volume (ml), tumor weight (g), viable (cells × 106 cell/ ml) and nonviable cell count (cells × 106 cell/ ml), median survival time (MST), percentage increase life-span (% ILS) and haematological parameters like RBC (cells106µl-1), WBC (cells × 103 µl-1) and HB content (g/dl) in DLA bearing mice.

Parameters	**Normal control (5ml/kg)**	**DLA Control(2x106cell/ ml)**	**DLA+MEVC (200mg/kg)**	**DLA+MEVC (400mg/kg)**	**DLA+5-FU(20mg/kg)**
Tumor Volume	-	5.06 ± 0.06	2.28 ± 0.11b,*	1.97 ± 0.12 b,*	0.59 ± 0.08 b,*
Tumor weight	-	4.42 ± 0.71	2.03 ± 0.11 b,*	0.68 ± 0.06 b,*	0.49 ± 0.02 b,*
Viable cell	-	9.85 ± 0.42	4.61 ± 0.21 b,*	1.90 ± 0.16 b,*	0.68 ± 0.03 b,*
Non	-	0.85 ± 0.14	1.09 ± 0.11 b,*	2.97 ± 0.11 b,*	3.91 ± 0.11 b,*
Viable cell	-	-	-	-	-
MST (days)	-	41.1 ± 0.32	31.72± 0.08	35.11 ± 0.40	45.11± 0.45
%ILS	-	00	52.02	68.455	86.18
RBC	9.08 ± 0.10	5.14 ± 0.32a*	3.03 ±0.24 b,*	4.15 ± 0.13 b,*	5.05 ± 0.24 b,
WBC	8.10 ± 0.01	15.04 ± 0.71 a*	10.39 ± 0.41 b,*	8.30 ± 1.1 b,*	6.01 ± 0.78 b,*
Hemoglobin	18.10 ± 0.01	9.21 ± 0.88 a*	6.18 ± 0.88 b,*	8.99 ± 0.55 b,*	9.86 ± 0.22 b,*

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Administration of MeVC at the doses of 200 and 400mg/kg significantly (P<0.01) decreased the tumor volume and viable cell count. Non viable cell count was significantly (P< 0.01) higher in MeVC treated animals comparing to DLA control animals. These results could connote either a direct cytotoxic affect of MeVC on tumor cells or an indirect local effect, which may involve macro-phage activation and vascular permeability inhibition.The prolongation of the animal life span was being considered as a reliable criterion for the depiction of efficacy of an anticancer agent6. Furthermore, the median survival time was increased to 30.7 ± 0.90 (%ILS = 42.87) and 37±0.39 (%ILS = 74.41) on administration of MeVC in a dose dependant manner. The rapid increase in ascitic fluid volume was observed in DLA bearing mice, ascetic fluid is the direct nutritional source for tumors growth it meets the nutritional requirements of tumor cells 12. The increase of life span of tumor bearing mice indicates reduction of nutritional fluid volume and seization of the tumor growth is a positive result and further determines the antitumor effect of MeVC. The major problems encountered in cancer chemotherapy are myelosuppression and anemia. The anemia exhibited in tumor bearing mice is mainly due to reduction of RBC or hemoglobin percentage and etiology is either due to iron deficiency or hemolytic/myelopathic condition 13.There was significantly (P<0.01) elevated level of WBC and significantly (P<0.01) reduced level of RBC and hemoglobin (Hb) in DLA control group as compared to normal control group (Table 1). But, treatment with MeVC at the doses of 200 and 400 mg/kg in DLA bearing mice significantly (P<0.01) increased both the RBC count and Hb content while WBC count was reduced significantly (P<0.01) when compared with the DLA control group.

IV. CONCLUSION

The present study results stated that the methanolic leaf extract of vitex negundo showed a significant in vitro and in vivo anti tumor activity against DLA cells. These important and significant preliminary finding can be taken as the basis upon which further studies should be carried out to delineate the detailed profile of these anti cancer actions of vitex negundo.

REFRENCES

[1]. Special Supplement on The Importance of Herbs by Jack Kittredge
[2]. Priya K, Krishankumari S, Vijaykumar M Cyathula prostrata: A potent source of anti cancer agent against Dalton's ascites in Swiss albino mice. Asian Pacific J Trop Med 2013; 6: 776-779.
[3]. Rajkumar V, Guha G, Kumar A Antioxidant and anti neoplastic activities of Picrorhiza kurroa extract. Food Chem Toxicol 2011; 49: 368-369.
[4]. Malcolm RA. Cancer: ENCYCLOPEDIA OF LIFE SCIENCES / Nature Publishing Group: 1-8, (2001).
[5]. M .Akhila , Kanakam Vijayabhaskar, P.Srinivasa Rao, Pharmacognostic estimation of powder and microscopic analysis of leaves stem of vitex castofolia: IJSR-Volume 9 Issue 1, January 2020.
[6]. Haldar PK, Bhattacharya S, Kar B, Bala A, Mazumder UK Chemoprventive role of Indigofera as palathoides in 20-methylcholanthrene induced carcinogenesis in mouse. Toxicol and Enviro Chem 2010; 92: 1749–1763.
[7]. Manojkumar P, Kochupappy RT, Subbuchettiaar G Synthesis of coumarin heterocyclic derivatives with antioxidants activity and in vitro cytotoxic activity against tumor cells. Actta Pharmaceutica 2009; 5: 159-170.
[8]. Patel S, Gheewala N, Suthar A, Shah A In vitro cytotoxicity activity of Solanum nigrum extract against Hela cell line and vero cell line. Int J of Pharm and Pceutical Sci, 2009; 1: 38-46.
[9]. Yoganarasimhan SN. Medicinal Plant of India Tamil Nadu. Regional Research institute Bangalore India, 2: 69-71(2000).
[10]. Bala A, Kar B, Haldar PK, Mazumder UK, Bera S Evaluation of anticancer activity of Cleome gynandra on Ehrlich's ascites carcinoma treated mice. J Ethno pharmacol 2010; 129:131–134 (2010).
[11]. D'Armour FE, Blood FR, Belden DA The Manual for Laboratory Work in Mammalian Physiology 3rd ed. The University of Chicago: Chicago 1965.
[12]. 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.
[13]. Kumar AS, Juvvadi RK, Kumar VK, Reddy M, Reddy V Effect of methanolic extract of Adenathera palvonina linn on Dalton's Ascitic Lymphoma. Indian J Res in Pharm and Biotech 2011; 1: 138-141.