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RESEARCH ARTICLE

ANTICANCER ACTIVITY OF VINCA ROSEA LINN ROOT EXTRACT AGAINST DALTON'S LYMPHOMA ASCITES (DLA) TUMOR CELLS IN MICE

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ABSTRACT

Objective: Vinca rosea Linn. is a commonly used plant for treating malaria, diabetes, and Hodgkin's lymphoma. The main objective of this study is to assess the antitumor activity of V. rosea roots in Dalton's Lymphoma ascites mice model. ***Methods:*** Antitumor activity of hydroalcoholic extract of V. rosea (HAEVR) roots is evaluated against Dalton's lymphoma ascites (DLA) tumor mice. After 24 hrs of tumor inoculation, the extract with doses 200 and 400 mg/kg is administered daily for 15 days. After administration of the last dose mice are sacrificed for observation of antitumor activity. Antitumor activity is assessed by monitoring the tumor size, cancer cell count, increase in body weight, haematological and biochemical parameters, and histopathological evidence. ***Results:*** The results evidenced significant results of HAEVR roots in the treatment control group against the above-mentioned parameters when compared to the mice of the DLA control group. ***Conclusion:*** The findings indicate that the HAEVR roots have antitumor activity against DLA-induced mice. Hence, it is evident that this extract could be a natural anticancer agent for human health.

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INTRODUCTION

Cancer stands as a significant global health hazard, claiming over six million lives annually. Comprising over 100 types of malignancies, cancer ranks among the primary burdens of chronic disease worldwide. Its multifactorial etiology renders it incredibly challenging to cure, underscoring the complexity of its pathogenesis[1].Plant-based substances have garnered significant attention lately due to their multifaceted applications. Medicinal plants are the richest bioresources of drugs of traditional systems of medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for syntheticdrugs[2].The utilization of plants and plant-derived products in cancer treatment is experiencing rapid growth within medical practices[3]. The Vinca rosea is a perennial tropical medicinal plant belonging to the family Apocynaceae. In Ayurveda, the V. rosea plant was used to treat many diseases, such as malaria, diabetes, and Hodgkin's lymphoma[4]. Medicinally, plant V. rosea plays a vital role as the plant is solely responsible for synthesizing monoterpenoid indole alkaloids. Among those alkaloids, two are very important alkaloids, namely, vinblastine (VBR) and vincristine (VCR). These two alkaloids are strong anticancer agents.

V. rosea has been used as a mouthwash and for curing ailments like scurvy, wounds, stomach-ache, and ulcers, to control haemorrhage, and as antioxidant, antimicrobial, anti-helminthic, anti-diabetic, anti-obesity and majorly anticancer activity[5].However, there is no scientific validation of Vinca roots being used against Dalton's lymphoma ascites tumour (DLA). Therefore, the present study evaluates the in-vivo anticancer activity of the roots of Vinca against DLA cells.

MATERIAL AND METHODS

Collection and preparation of Vinca root extract: The Vinca rosea Linn. plant was diligently identified, and its roots were gathered from local areas in Varanasi. Taxonomic verification of the plant was conducted by experts at the Department of Dravyaguna, Banaras Hindu University, Varanasi, UP. Upon collection, the roots underwent thorough cleaning with tap water to remove any impurities. They were then carefully dried in a well-ventilated environment, shielded from light, at room temperature to preserve their integrity. After complete drying, the roots were finely ground into a powder-like consistency. This powder was stored in clean, airtight plastic containers, ensuring they were shielded from light, heat, and moisture until needed for further

experimentation. The powdered drug was carefully extracted with a hydroalcoholic solvent (50:50) using the Soxhlet apparatus. Subsequently, the filtrate obtained underwent lyophilization for a period of 2-3 days to yield a powdered extract. This precisely prepared hydroalcoholic extract sourced from Vinca roots was specifically selected for use in the in vivo study.

Selection of Animals: Male and female adult Swiss Albino mice, approximately 8-9 weeks old, with an average weight ranging from 25 to 30 grams, were obtained from the Central Animal House at the Institute of Medical Sciences, Banaras Hindu University, Varanasi. Prior to the experiment's commencement, the mice underwent a seven-day acclimatization period in the departmental animal facility. During this acclimatization period, the mice were segregated by gender and housed in poly acrylic cages, with no more than six animals per cage. They were kept in a controlled environment with a temperature maintained at 25 ± 2°C and a 14/10-hour dark/light cycle. Throughout the acclimatization and experimental periods, the mice had unrestricted access to standard laboratory diet and water ad libitum. The study was conducted only after obtaining clearance from the Institutional Animal Ethical Committee (Approval No: 542/GO/ReBi//S/02/CCSEA), ensuring compliance with ethical guidelines and regulations regarding the use of animals in research.

Acute Toxicity Study: The acute oral toxicity evaluation of Vinca root extract in Swiss albino mice was carried out in accordance with OECD guidelines 425[6]. Prior to dosing, the animals underwent an overnight fasting period while maintaining access to water. Subsequently, a single oral dose of 2000 mg/kg body weight was administered to five animals, and their mortality was observed over a 14-day period. The extract demonstrated safety up to the dose of 2000 mg/kg body weight. To calculate effective doses, which typically range from 1/5th to 1/10th of the lethal dose, doses of 200 and 400 mg/kg body weight were selected for further study[7].

In Vivo Anticancer activity of Vinca rosea roots Hydroalcoholic extract on DLA in Mice

Induction of Cancer using Dalton's Lymphoma Ascites Cells: DLA cells were borrowed from the Department of Zoology, Banaras Hindu University, Varanasi. The cells were sustained in vivo within Swiss albino mice through intraperitoneal administration. As the tumour cells were transferred into the grouped animals, the DLA cells were withdrawn from the peritoneal cavity of the mice using saline. The cells were counted, and further dilution was made so that the total cells should be 50,000 cells/mland this dilution was given intraperitoneally.

Animal Grouping and Treatment Schedule: The animals were divided into five groups, with each group comprising 12 mice (6 male and 6 female). All mice, except for those in Group I (Normal Control), received a 1 ml cell suspension of DLA tumor cell line intraperitoneally. Group I served as the Normal Control and received normal saline (5 ml/kg).

Group II served as the DLA tumor cell line control. Group III, the Standard control group, received the standard drug 5-fluorouracil at a dose of 10 mg/kg body weight. After 24 hours of tumor inoculation, the treatment groups IV and V received Vinca root extract orally once daily at doses of 200 and 400 mg/kg body weight, respectively. On day 15, all mice were subjected to an 18-hour fasting period, after which blood samples were collected via direct cardiac puncture for the determination of hematological and biochemical parameters. Subsequently, the animals were sacrificed for the assessment of antitumor parameters[8]. The antitumor activity of Vinca root extract was evaluated by observing changes in the following parameters[9]**:**

Tumour volume and weight: The mice underwent dissection, and the ascitic fluid was collected from the peritoneal cavity. The volume of the fluid was measured by taking it in a graduated centrifuge tube (ml).

Tumour weight was measured by taking the difference in weight of the mice before and after the collection of the ascitic fluid from the peritoneal cavity (g)to assess the effectiveness of the treatment in reducing tumor growth.

Body weight: The average increase in body weight was determined by assessing the weight of mice from the beginning to the 15th day of the studyto monitor any changes or effects resulting from tumor induction and treatment interventions.

Assessment of tumour cell count: The ascitic fluid (0.1 ml) was withdrawn from the peritoneal cavity of each mouse by sterile syringe and diluted with 0.8 ml of sterile Phosphate buffer solution (PBS) and 0.1 ml of Trypan blue (0.1 mg/ml). The total number of living cells was counted using a Haemocytometer. Quantification of tumor cells is done to determine the extent of tumor regression or inhibition following treatment.

Haematological parameters: The blood collected from mice was used for the estimation of haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet count, and packed cell volume (PCV) by standard proceduresto assess the impact of the treatment on hematopoietic function[9].

Biochemical parameters: Analysis of blood biochemical markers such as liver enzymes (ALT, AST), kidney function markers (creatinine, bilirubin), to evaluate the effects of the treatment on organ function and tumor progression.

Histopathological parameters: At the end of the experiment (15th day) animals were sacrificed and body organs (Heart, Liver, and Kidney) were excised as well as examined microscopically for any changeto assess histopathological changes, tumor morphology, and cellular response to treatment. Relative organ weight was assessed by absolute organ weight (g) × 100/ body weight of mice on the day of sacrifice (g)to evaluate any treatment-related toxicity or organ-specific effects. These parameters collectively provide valuable insights into the antitumor efficacy and potential toxic effects of Vinca root extract.

Statistical analysis: The values are represented as mean ± SD. The experimental data was assessed by a one-way Anova / Kruskal-Wallis test followed by a post-hoc test/ Mann-Whitney test comparison. The results were statistically significant when the p-value was< 0.05.

RESULTS AND DISCUSSION

Effect on Tumor Growth: Tumour growth in DLA tumour-bearing mice was assessed by parameters such as tumour volume, tumour weight, and cell count. The tumour volume, tumour weight and cell count were found to be significantly ($p<0.05$) increased when compared to the normal control mice. Administration of HAEVR at doses 200 and 400 mg/kg significantly ($p<0.05$) decreased the tumour size and cell count as compared to the DLA control group (Table 1). Many studies have reported the action of Vinca rosea against cancer cells.

Effect on Biochemical Parameters: In the present study, the biochemical examination of DLA-inoculated animals showed only slight variations but was not found significant ($p > 0.05$). No significant changes were observed in the treatment control group with dosesof 200 and 400 mg/kg (Table 3).

Markedly elevated serum ALP, or hyperalkaline-phosphatasemia, is predominantly associated with specific disorders such as biliary cirrhosis, hepatic lymphoma, and sarcoidosis[13]. DLA ascites tumour did not evidence any significant changes in the biochemical profile of the mice.

Table 1. Effect of HAEVR root on mice body weight, tumour size and cancer cell count

Groups	Increase in Body weight	Tumour vol. (ml)	Tumour wgt.(g)	Cell count
G_1	26.75± 0.86	-	-	-
G_2	40.50±4.70**	8.16 ±2.26**	8.65 ±2.28**	5.15±0.67**
G_3	39.00±2.13***	3.83±0.42***	4.06±0.47***	0.85 ±0.40***
G_4	35.50±1.31***	5.53±0.47***	5.48±0.47***	1.23±0.44***
G_5	35.67±1.87***	4.13 ±0.41***	4.35±0.33***	1.02±0.50***

G_1-Normal saline control, **G_2**-Cancer control, **G_3**-Standard control**, G_4**-Treatment control HAEVR (200 mg/kg), **G_5**-Treatment control HAEVR (400 mg/kg); All values are expressed as mean ±SD (n = 12). Data was assessed by the one-way Anova method followed by Post hoc test.
** - Values are significantly different from normal control (G_1) at $p < 0.05$.; *** - Values are significantly different from Cell line control (G_2) at $p < 0.05$. HAEVR – Hydroalcoholic extract of Vinca rosea.

Table 2. Effect of HAEVR root on Hematological parameters

Groups	Hb (gm/dl)	RBC count (Mill/cumm)	Total WBC (cells/ml × 10^3)	Platelets (/cumm)
Normal saline (5 ml/kg)	12.83 ±0.96	5.28 ±1.03	11.66 ±0.58	861.67 ±67.74
DLA control (50,000 cells/mice)	7.84 ±1.23**	3.79 ±1.55**	28.63±8.20**	378.83±196.28**
DLA + 5-Fu (10 mg/kg)	11.58±1.06***	6.06±1.02***	12.70±1.48***	662.67±128.12***
DLA+HAEVR(200 mg/kg)	11.48±1.28***	4.21±1.01***	13.56±1.44***	615.50±135.12***
DLA+HAEVR(400 mg/kg)	10.95±0.84***	3.34 ±1.13***	11.81±1.15***	719.33 ± 70.06***

All values are expressed as mean ±SD (n = 12). Data was assessed by the one-way Anova method followed by Post hoc test.
** - Values are significantly different from normal control (G_1) at $p < 0.05$. *** - Values are significantly different from Cell line control (G_2) at $p < 0.05$. HAEVR – Hydroalcoholic extract of Vinca rosea.

Table 3. Effect of HAEVR root on Biochemical parameters

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (g/dL)	Bilirubin (mg/dL)	Creatinine (mg/dL)
Normal saline (5 ml/kg)	164.83±44.35	17±2.41	22.67±6.67	2.58±0.36	0.15±0.12	0.28±0.20
DLA control (50,000 cells/mice)	147±48.24	21±5.46	31±8.46	2.48±0.37	0.16±0.12	0.33±0.24
DLA+5-Fu (10mg/kg)	171±70.60	13.17±2.36	19.50±3.89	2.71±0.49	0.09±0.02	0.30±0.18
DLA+HAEVR (200 mg/kg)	156±88.23	18.50±6.37	21.67±4.69	2.86±0.45	0.18±0.11	0.21±0.09
DLA+HAEVR (400 mg/kg)	128.17±37.11	16.83±4.76	20.33±9.81	3±0.27	0.30±0.26	0.18±0.07

All values are expressed as mean ±SD (n = 12). Data was assessed by the one-way Anova/Kruskal-Wallis method followed by Post hoc/ Mann-Whitney test. Values were not found significant between the groupswith $p > 0.05$. HAEVR – Hydroalcoholic extract of Vinca rosea.

Effect on Hematological Parameters: Hematological parameters exhibited significant alterations ($p < 0.05$) after the 14th day of treatment when compared to the DLA control group. Total WBC count increased in the DLA control group, while Hemoglobin, RBC, and platelets decreased in this group compared to the normal group. Treatment with HAEVR root at doses 200 and 400 mg/kg significantly increased the Hemoglobin, RBC and platelets and decreased the WBC count to about normal level (Table 2). However, these parameters were better controlled by standard control drug. Anemia or myelosuppression is commonly observed in ascitic lymphoma. The anemia encountered in ascitic lymphoma is primarily attributed to iron deficiency, stemming from either hemolytic or myelopathic conditions, ultimately resulting in reduced RBC count or hemoglobin percentage[12].Therefore, treatment with HAEVR root extract replenishes the hemoglobin percentage, RBC, and WBC count to normal levels, providing evidence of its protective action on the hematopoietic system.

Effect on Histopathological Parameters: After the sacrifice of mice, immediately Heart, liver, and kidney organs were immediately fixed in 10% buffered formalin and examined employing routine histological techniques[14]to observe the pathological changes under the light microscope. Sections of the heart, liver, and kidney wereobserved with normal microscopic characteristics with few dilated and congested blood vessels and necrosis inthe focal area of the centrihepatic portion ofthe liver.

Also, the heart shows few dilated blood vessels between the myositis with loss of striations in fibres in DLA control group mice. The relative organ weight of the heart, liver, and kidney was assessed for any changes. A significant ($p < 0.05$) increase in the size of organs was observed in all groups with DLA tumour cells.

CONCLUSION

The current study sheds light on the anticancer activity of HAEVR root extract against DLA-induced Swiss albino mice. Dalton's ascites lymphoma is a transplantable poorly differentiated malignant tumour that initially manifests as lymphocytes in mice, growing in both solid and ascitic forms. The findings revealed a decrease in cancer cell counts, providing confirmatory evidence of protection against DLA cells. Consequently, this study implies significant in vivo antitumor activity of Vinca rosea roots.

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