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Anticarcinogenic and cytotoxic potential of *Hemidesmus indicus* root extract against Ehrlich Ascites tumor

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ABSTRACT

The purpose of the present work was to evaluate anticarcinogenic and cytotoxic potential of Hemidesmus indicus methanolic root extract (HiRe) against Ehrlich Ascites Tumor. The extract showed a significant in vitro cytotoxic activity against Ehrlich Ascites Tumor (EAT) cell line. IC_{50} value for EAT cell line was 274.83µg.The anticarcinogenic activity of the extract was determined by using EAT cell line induced ascites tumor model in mice and its comparison with standard anticancer drug cyclophosphamide. The treatment with methanolic root extract of Hemidesmus indicus (50 mg/kg and 100 mg/kg body weight) significantly increased the body weight of ascites tumor model. The life span of treated animal was increased up to 67.78%.The results were more significant in mice treated with 100 mg/kg body weight. This study revealed that Hemidesmus indicus may have a great potential to be exploited for the search of anticancer drugs.

Key word: cytotoxicity; cyclophosphamide; *Hemidesmus indicus*; *anticancer*

INTRODUCTION

Over the past few years, cancer has reminded a major cause of death and the number of individuals living with cancer is increasing. Cancer is mostly treated by chemotherapy, radiation therapy and surgery, but each treatment has its own side effects. Cytotoxicity is one of the themotherapeutic targets to anticarcinogenic activity [1]. Most of the clinically used anticarcinogenic agents possess significant cytotoxic activity in cell culture system. Cyclophosphamide is a cytotoxic alkylating drug with a high therapeutic index against a variety of cancers [2]. Its side effects include nausea, vomiting, and bone narrow suppression. Hence a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets [3]. Herbal remedies are natural products derived from plants and plant extracts have been used traditionally to treat various diseases or to promote general health [4].The search for herbal remedies and natural substances and understanding their mechanisms of action in the body is on the rise. Due to the enormous property of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Indian medicinal plants are quoted to be useful in different types of cancer diseases [5, 6]. One such plant is *Hemidesmus indicus Linn.* belongs to the family (Apocynaceae) [7], commonly referred to as Indian sarsaparilla, Anantamool or Nannari is a commonly available perennial climbing plant, used as the main ingredient in the preparation of the cool and refreshing drink Nannari sherbat. It is a native of India and also found in south tropical Asian countries such as Pakistan and Sri Lanka [8]. *Hemidesmus indicus* is a well known medicine for antioxidant and anti-inflammatory diseases [9].Tribal people used this plant to treat the cancers of abdomen and skin. The root decoction of *Hemidesmus indicus* R.Br. was tested on hepatoma HepG2 cell lines. [10,11]. The plant is used in traditional medicine in biliousness, respiratory disorders, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite and burning sensation [12,13]. The major chemical constituents are Coumarin, hemidesmine, hemidine, hemidesine and rutin [14].With all these wide spectrum of

medicinal properties, the present study aims to evaluate the anticarcinogenic and cytototoxic activities of methanolic root extract of *Hemidesmus indicus* against EAT cell line, and comparison with standard chemotherapeutic agent cyclophosphamide.

MATERIALS AND METHODS

Collection of plant material and Preparation of crude extract

Hemidesmus indicus was collected from Authenticated crude drug supplier in Mysore and identification was done by a taxonomist. Identification was confirmed by depositing the voucher specimens in the Herbarium of the Department of Botany, University of Mysore, and Mysore. The powdered shade dried plant roots were exhaustively extracted with methanol using soxhlet extraction apparatus. The solvent of extraction was evaporated to dryness and the residue thus obtained was used for anticarcinogenic and cytotoxic analysis.

Animals

Swiss albino mice were obtained from animal house, Department of Zoology,
University of Mysore. Mysore, India. They were kept under standard conditions of humidity and temperature in animal house of Department of Zoology.

Cell line

Ehrlich Ascites Tumor (EAT) cells were obtained from National Center for Cell Science, Pune, India. The cell was maintained in mice by intraperitonial inoculation.

In vitro cytotoxic activity

Short-term cytotoxic activity of the HiRe was assayed by determining the percentage of viability of EAT cell using the trypan blue dye exclusion method with haemocytometer [15]. EAT cells were cultured in the peritoneal cavity of healthy albino mice weighing between 28 to 30g by injecting a suspension of cells (1 x 10^{10} cells / ml) intra peritoneal. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15 and washed with phosphate buffered saline (PBS), (0.2 M, pH 7.4) and centrifuged for 15 min at 1,500 rpm in a centrifuge. The pellet was re-suspended with PBS and the process was repeated three times. Finally, the cells were suspended in a known quantity of PBS and the cell count was adjusted to 1 x 10^{10}cells / ml. Then, 0.1 ml of this diluted cell suspension was distributed into test tubes and exposed to 0.1 ml each of the different concentrations of the HiRe and incubated at 37^{o}C for 2 h. After 2 h, the trypan blue dye exclusion test was performed to determine the percentage viability and the IC_{50} value was calculated.

Effect of HiRe on the survival rate of ascites tumor bearing animals

Animals (Male, 6-8 weeks old) weighing 28-30 g were divided in to four groups of six animals each. Viable EAT cells (1 x 10^{10}) in 0.1 ml of phosphate buffered saline (PBS) were injected in to the peritoneal cavity. Group 1, control: Oral administration of 0.1 ml of distilled water / animal. Group 2, Standard: Cyclophosphamide 10mg/ kg body weight. Group 3, HiRe 50 mg/ kg body weight. Group 4, HiRe 100mg/kg body weight. Drug was administered orally and cyclophosphamide was given by intraperitoneal injection from the first day of tumor induction. The death pattern of animals due to tumor burden was noted and the percentage increase in life span (% ILS) = [T-C/C] x 100 where 'T' and 'C' are mean survival of treated and control mice respectively. Percentage increase in body weight % IBW = [W-W_o/W_o] x 100 where W_o was the average initial weight of mice before inoculation of EAT cell lines and W was the average weight of mice on the fifteenth day of inoculation.

Statistical analysis

All data are expressed as mean ± standard deviations (SD).Significance of differences was assed at $P<0.05$ by one way ANOVA followed by post hoc Dunnet's test.

RESULTS AND DISCUSSION

Cancer is a disease of misguide cells that have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest cause of death in the world. Of all the available anticancer drugs during 1940-2002, 40% were natural products or derived from natural product, with another 8% being natural product mimics [16].Chemotherapy is one of the methods for the treatment of cancer. A major complication of chemotherapy is its toxicity to normal cells, which is due to the inability of drug to differentiate between normal cells and malignant cells. This often impacts the efficacy of the treatment and even makes it impossible to cure the patients. One of the requisites of cancer chemo preventive agent is elimination of damaged or malignant cell through cell cycle inhibition or induction of apoptosis with less or no toxicity to normal cells [17].

Cancer chemoprevention, utilizing chemical compounds or natural products revert or inhibit malignant cell transformation, prevents invasion, metastasis would be less painful, more economical and rational approach for cancer control .The use of herbal medicine or dietary agents is being increasingly utilized as an effective way for the management of many cancer treatments [18]. The greatest recent impact of plant derived drugs is observed in the area of antitumor research, where compounds such as taxol, vinblastine, vincristine, and camptothecin have dramatically improved the effectiveness of chemotherapy against some of the dreaded cancers [19]. Hence, there is a great potential for the development of anticancer drugs from the essentially untapped reservoir of the plant kingdom. Here the methanolic extract of HiRe showed marked cytotoxic activity (Fig. 1) for EAT cell lines. The concentration required for 50% death (IC_{50}) was found to be 274.83for EAT cells.

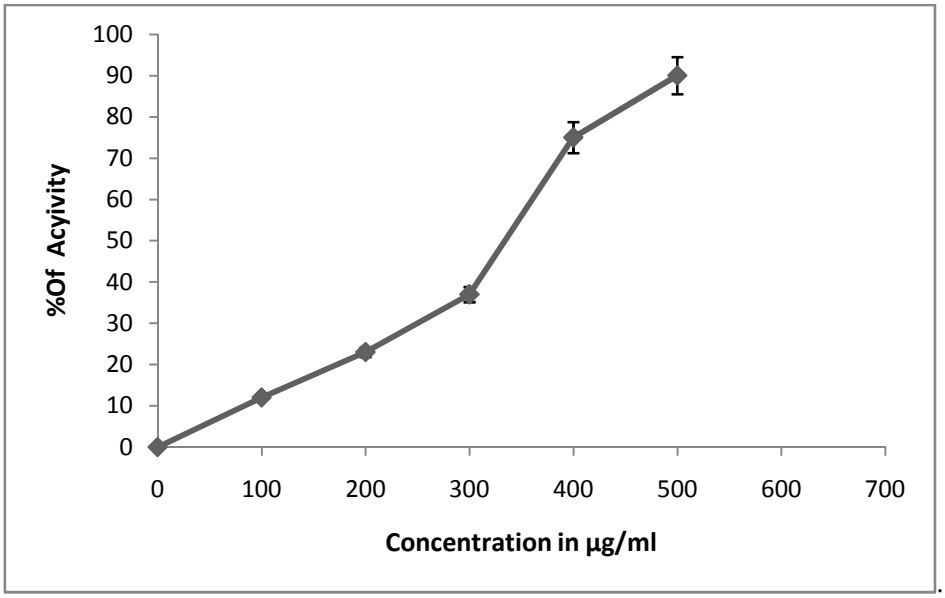

Figure.1 Cytotoxic activity of methanolic extract of *Hemidesmus indicus* on EAT cell lines: Dose response curve.

The animals of the tumor control group inoculated with EAT survived for a period 18±1.58 days. The treatment with cyclophosphamide survived for 30±1.58 days, the HiRe at 50 and 100 mg/kg body weight increased the average life span of animals by 24.8±1.3 days and 30.2±2.68 days, respectively (Fig.2)

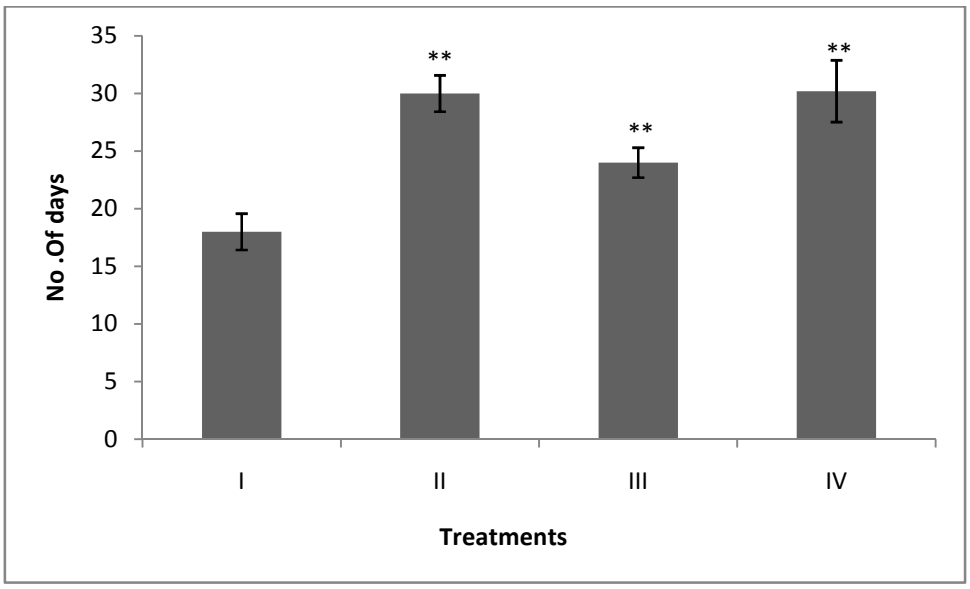

Figure.2 Effect of methanolic extract of HiRe on average life span of ascites tumor bearing mice. Groups- I: Control, II: Cyclophosphamide 10mg/kg, III: HiRe 50 mg/kg, IV: HiRe 100 mg/kg. ** Represents comparison between control and treated groups.

The increases in life span at 100 mg / kg body weight were found to be significant (P<0.001). The HiRe at the 100mg /kg body weight dose was found to be more potent in inhibiting the proliferation of EAT with the percentage increase in life span of 67.78%, cyclophosphamide 10mg/kg, 66.67%, HiRe 50mg/kg 38.8%.

The percentage increased in body weight was reduced in HiRe treated animals in a dose depended manner with significance of (P <0.01) respectively (Fig.3 and 4).

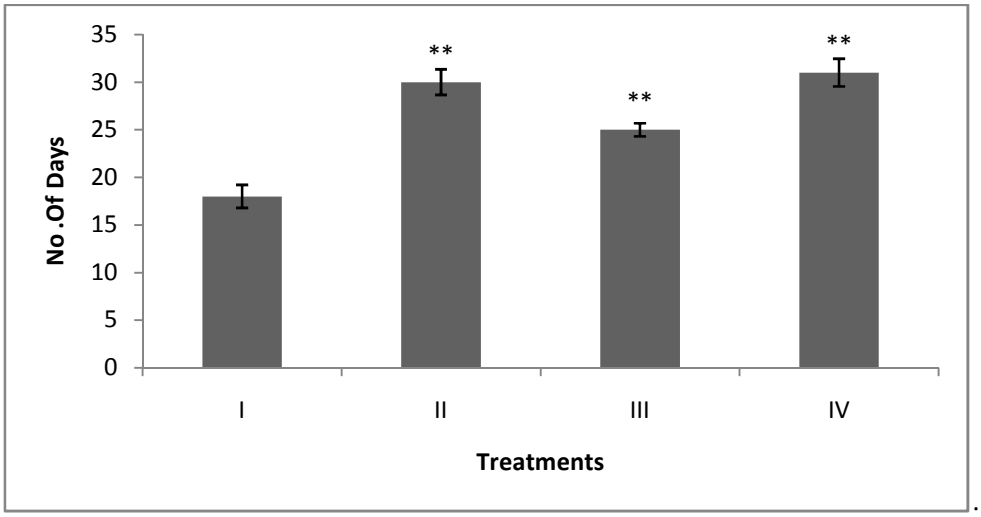

Figure.3 Effect of methanolic extract of HiRe on average body weight of ascites tumor bearing mice. Groups- I: Control, II: Cyclophosphamide 10 mg/kg, III: HiRe 50 mg/kg, IV: HiRe 100 mg/kg. ** Represents comparison between control and treated groups.

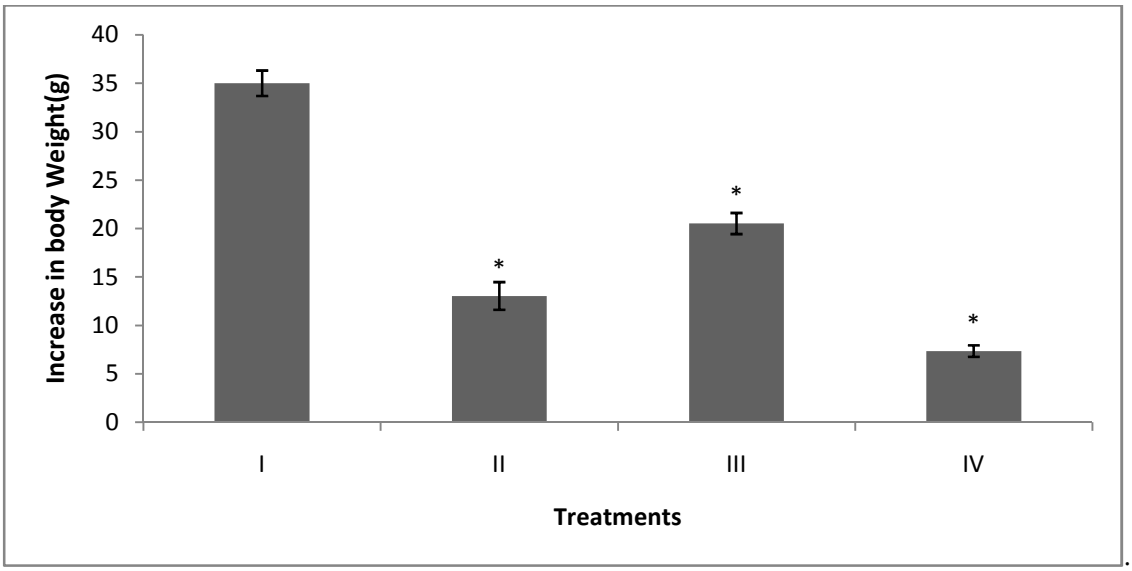

Figure.4 Effect of methanolic extract of HiRe on increase in body weight of ascites tumor bearing mice. Groups- I: Control, II: Cyclophosphamide 10mg/kg, III: HiRe 50 mg/kg, IV: HiRe, 100 mg/kg.* Represents comparison between control and treated groups.

Ehrlich ascites tumor is a rapidly growing carcinoma with very aggressive behaviour [20]. It is able to grow in almost all strains of mice. The Ehrlich ascetic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascetic fluid formation[21] .The ascetic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [22]. The results of present study proved that HiRe can reduce the ascites tumor burden in a dose depended manner. HiRe 100mg / kg body weight is more effective than the standard drug cyclophosphamide at a dose of 10mg / kg body weight. This could indicate either a direct cytotoxic effect of HiRe on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition.

CONCLUSION

Our results showed that HiRe can reduce the ascites tumor burden in a dose depended manner. HiRe 100mg / kg body weight is more effective than the standard drug cyclophosphamide at a dose of 10mg / kg body weight. The percentage increase in life span at the 100 mg / kg body weight dose of the HiRe was found to be the highest among the two doses tested. These results provide strong evidence suggesting that HiRe have in vitro cytotoxicity against EAT cell lines. The cytotoxic activity of HiRe against EAT cell line partially explains its significant anticarcinogenic activity against ascites tumor. These results are leading us to the hypothesis that *Hemidesmus indicus* root extract could be used to develop effective therapeutic approaches towards the prevention or treatments of various immune conditions and different types of cancer.

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