A STUDY ON ANTI-CANCER ACTIVITY OF EUPHORBIA NERIIFOLIA (MILK HEDGE) LATEX

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Abstract - The study was carried out to evaluate antitumor activity of extracts of latex of *Euphorbia neriifolia* in validated experimental animal models. Antitumor activity of acetone extract was investigated against DLA induced ascites tumor in mice. Two doses 50 mg/kg and 100 mg/kg b.w p.o of the extract were subjected for the evaluation of Antitumor potential against DLA induced mice and Cyclophosphamide used as standard. The parameter percentage mortality was studied. Phytochemical study and Invitro cytotoxicity studies were also performed. It was observed that Both EN- 50 and EN- 100 showed dose dependent significant reduction in mortality rate and increased recovery in the extract treated mice when compared with vehicle control. Maximum protection was observed at 100mg/kg. Terpenoids were found to be the major component of acetone extract. The present study concluded that latex extract of *Euphorbia neriifolia* has significant antitumor potential. The exact mechanism for the antitumor activity of EN is still unknown hence further studies are needed to isolate, characterize the active principles and to find out the exact mechanism responsible for its antitumor activity.

Keywords - Tumor, Acetone, Cyclophosphamide, *Euphorbia neriifolia.*

I. INTRODUCTION

Cancer is the name given to a collection of related diseases where some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start to develop almost anywhere in the human body, which is made up of trillions of cells. Normally, the human cells divide to form new cells as the body needs and die getting replaced by other new cells. However, when cancer develops this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed[1]. These extra cells can divide without stopping and may form growths called tumors. Since, the definitive target for all these factors is mostly genetic material, cancer can be considered as a genetic disease[2]. Being a complex group of diseases, Cancer is supposed to be caused by both internal (genetic mutations, harmones, immune conditions, etc) and external factors (chemical reagents, ionizing radiations, tobacco, etc).

Cancer needs to be treated at the earliest and chemotherapy and surgery have always been the standard methods for the treatment of cancer, although not been fully effective. Even though some progress has been made in cancer diagnosis and treatment, the high incidence and low survival rate of patient have still been reported[3]. Now a day's many synthetic and chemotherapeutic agents have been developed for the treatment of tumour but they show various types of side effects like alopecia, skin eruptions, reduced immunity, secondary carcinogenesis and some of the more common side effects are low blood counts that increases the possibility of developing infection or anaemia, tiredness, mouth soreness, nausea, vomiting, loss of appetite, constipation or diarrhoea, hair loss ,skin changes or reactions, pain or nerve changes, changes in infertility and sexuality etc[4]. Hence, to make the course of treatment more convenient, herbal drugs have been developed because they are not known for severe side effects. The development of new therapeutic approach remains one of the most challenging in cancer research. Medicinal herbs have started moving from border to mainstream use with a greater number of people in search of remedies and health approaches free from side effects caused by synthetic chemicals[5].

Euphorbiaceae, the spurge family, comprises some 7,500 species and 275 genera of flowering plants distributed primarily in the tropical regions[6]. The largest genus of family Euphorbiaceae is Euphorbia with about 1600 species. It is characterized by the presence of white milky latex that exudes when broken and which is more or less toxic[41]. *Euphorbia neriifolia* (Indian Spurge tree, Hedge Euphorbia), is one of the different species of Euphorbia genus plants, with wide range of local medicinal uses throughout the areas in which it is grown. There is a claim that fresh latex of *Euphorbia Neriifolia* can be used in the treatment of Tumour[7]. Hence, the latex of *Euphorbia neriifolia* is used in the present study.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

The latex of the plant Euphorbia neriifolia was collected from Panjya, Mangalore in February 2016. Immediately after the collection Toluene was added as it acts as a preservative and were stored in ambet color glass bottles. The extraction was carried out using acetone as a solvent in the magnetic stirrer. After extraction the product was centrifuged, filtered and dried at room temperature.

2.2. Invitro Cytotoxicity Studies

The invitro cytotoxicity studies were carried out in both the extracts and using two cell lines: EAC cell lines and DLA cell lines. Short term cytotoxicity analysis of both the extracts (Acetone and Methanolic extract) was assayed by the percentage viability of both the DLA and EAC cells using Trypan blue exclusion method. Tumor cells were aspirated from the peritoneal cavity of Tumor bearing mice, added to test tube containing PBS, and washed with PBS for 3 times.

- Cells were suspended in 1ml PBS and adjusted the cell number to $1_x\ 10^6$ cells/ml.
- Then viability of cells was checked using Trypan blue stain (0.1ml cell sample + 0.8 ml PBS + 0.1ml Trypan blue) with a haemocytometer.
- The cells were then incubated at 37ºC for 3 hours with different concentrations of drug (10-50µg) with $1_x\ 10^6$ tumor cells.
- After incubation, 0.1ml of Trypan blue, were added and determined the number of dead cells using haemocytometer.

$$\% \text{ of cytotoxicity} = \frac{\text{Number of dead cells}}{\text{Number of total cells}} \times 100$$

2.3.In Vivo Antitumor Study

Induction of Tumor in animals: DLA and EAC cells were aspirated from the peritoneal cavity of mice, washed with PBS and 0.1 ml cell suspension containing 10^6 cells were used for injection. Ascites tumor was developed by injecting $1_x\ 10^6$ EAC or the DLA cells intraperitonially to ascites of Swiss albino mice.

Ascites tumor: Injecting EAC cells into the intra peritoneal cavity of mice produced ascites tumor. The animal grouping and drug administration followed.

The animals were grouped into 5 with 6 animals each.

Group 1: Control.

Group 2: Standard (10 mg/kg body weight of Cyclophosphamide).

Group 3: Vehicle control (200µl of Propylene glycol).

Group 4: Drug treated (Low dose = 50mg/kg).

Group 5: Drug treated (High dose = 100mg/kg).

The drug treatment had been started the next day after the induction of the tumor for 10 days. The animals were observed for the development of the tumor and death due to tumor burden was recorded for 32 consecutive days. The life span of the animals were calculated using the formula,

$$\% \text{ Increase in life span (ILS)} = \frac{(T-C)}{C} \times 100$$

Where T and C are mean survival of treated and control mice.

2.4.Thin Layer Chromatography

Solvent system used: Toluene: Ethyl acetate: Formic acid (7:3:0.3).

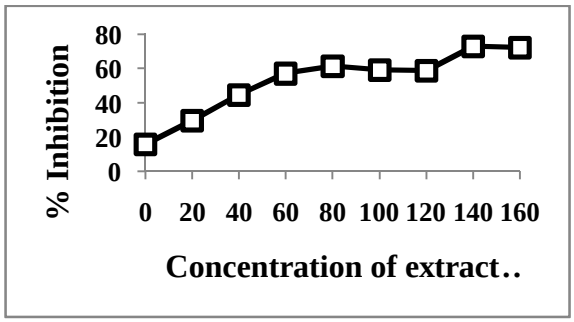
Stock solution of two extracts: Stock solutions of methanolic extract and Acetone extract was prepared by dissolving 10mg of extract in 1ml of methanol and ethanol respectively. Solvent system was allowed to move through the stationary face which was spotted with the sample and the R_f values were calculated using the formula:

$$R_f \text{ value} = \frac{\text{Distance moved by the solute front}}{\text{Distance moved by the solvent front}}$$

The bands were observed under short wave UV and the plate was tested with different spraying reagents, for Alkaloids, Terpenoids, Flavonoids, amines and Phenols.

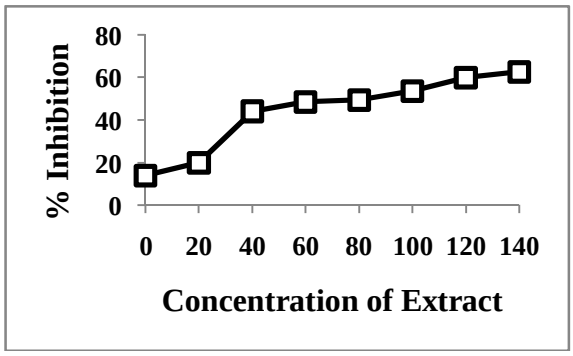
III. RESULTS AND DISCUSSION

3.1.Invitro Cytotoxicity Studies Of Acetone Extract For DLA Cell Lines And EAC Cell Lines



IC_{50} = 51 +/_ 3.6µg/ml

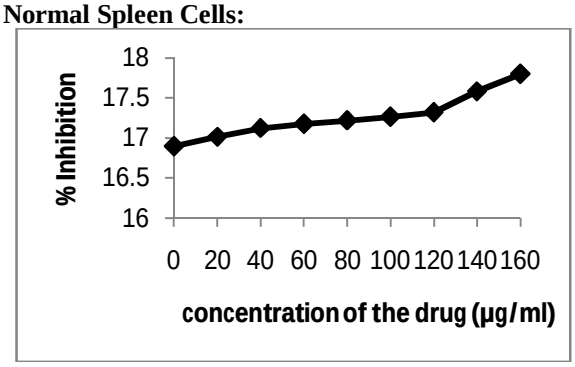
Graph 1: Invitro cytotoxic activity of Acetone extract on DLA cell lines.



IC_{50} = 82 +/_ 1.2 µg/ml

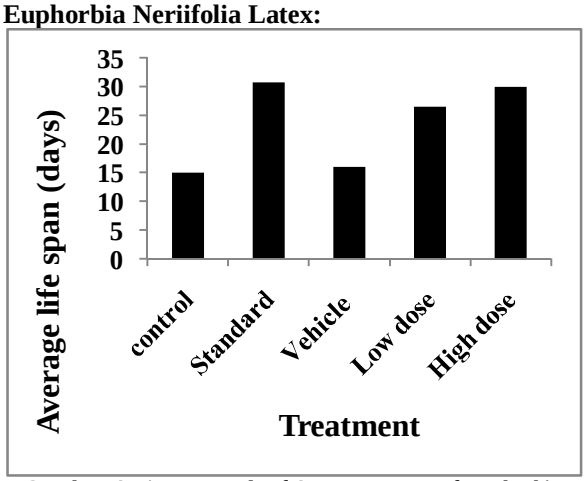
Graph 2: Invitro cytotoxic activity of Acetone extract on EAC cell lines.

3.2.Effect Of Crude Acetone Latex Extract On Normal Spleen Cells:



Graph 3: Invitro cytotoxic activity of Acetone extract on normal spleen cells.

3.3. Antitumor Effect of Acetone Extract of Euphorbia Neriifolia Latex:



Graph 4: Antitumor study of Acetone extract of *Euphorbia neriifolia*.

The acetone extract showed positive result for Terpenods, Phenols and Amines in TlC.

The acetone extract was screened for its cytotoxic activity against the cancer cells, which was found to contain terpenoids as the major component, it showed toxicity. But the methanolic extract did not show any kind of cytotoxicity towards the cancer cells [11]. Hence, the acetone extract was used for invivo antitumor study and was successful in showing antitumor activity. On DLA cells the leaf extracts showed an increase in cytotoxicity with increase in dose. The IC50 value of the Acetone extract of the latex of the EN was 51µg/ml and IC_{50} value of methanolic extract was insignificant. On EAC cells the Acetone extract of latex of EN had an IC50 value of 82µg/ml and IC_{50} of methanolic extract was again found to be insignificant.

Similarly, the IC50 values for EN latex extract on splenocytes of rats had been performed and the cytotoxicity was minimal in the acetone extract and was found to be more than 100 µg/ml above which it is considered to be insignificant. Tumor bearing animals treated with lower dose of 50mg/kg and higher dose of 100 mg/kg of acetone extract of EN showed a significant increase in survival period of 26.5 and 30 days when compared to control animals without any treatment having a survival period of nearly 15 days and the vehicle control treated with the propylene glycol with a survival period of 16 days.

Euphol, which is a terpenoid has been proved to have anticancer activity. Through literature review, it was known that Euphol is a terpenoid and is present in EN latex. Hence, Euphol can be an agent responsible for the antitumor activity of acetone extract of EN latex[11].

As the extract used for the study was a crude one, it is not possible to point out that the particular compound is responsible for anticancer properties shown by the plant. It may be better assumed that the biologically active principles present in the extract act in synergestic fashion to inhibit the properties studied in the present work. The cytotoxic activity of E. neriifolia against the DLA and EAC cell lines partially explains its significant antitumour activity against the ascites tumor.

All the data obtained from the study are strictly by the techniques performed under laboratory conditions. Further investigation will be required to get the complete picture out of it, which might change the face of this dreadful disease of present society, Cancer.

CONCLUSION

The present study conclude that the acetone extract of Euphorbia neriifolia has significant potential anticancer activity. All the results clearly indicated that the crude latex extract has remarkable capacity to inhibit the growth of ascites tumor induced by DLA cell line in the experimental animals. Perhaps, the presence of cytotoxic components of the latex might be imparting the anti cancer efficacy. The exact mechanism for the anticancer activity *euphorbia neriifolia* latex is still unclear. Further studies are needed to isolate the bioactive principles responsible for anticancer activity and to determine the exact mechanism of action.

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