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Research Article

CYTOTOXIC INFLUENCING ACTIVITY OF LATEX OF *EUPHORBIA ANTIQUORAM* LINN

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ABSTRACT

Cytotoxicity measurements were originally designed for rapid and inexpensive analysis of soluble pharmaceuticals. The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Mouse spleen cells have been successfully used to analyze mechanisms of cytotoxicity for a variety of anticancer drugs. The results in BSLA showed that the percentage of lethality rate of the shrimps was found to be less which indicates that at low concentration the survival rate was increased and protected from the effects of test sample. The calculated LC_{50} value for the tested sample was 20µg. The percentage of viable cells was assessed by MTT assay in a time and dose dependent manner in mouse spleenocytes and LC_{50} value of methanolic extract of latex milk was found to be 20µg at different time intervals. SRB and Neutral red assay showed that latex milk is safe to normal spleen cells.

Keywords: Apoptosis, *Euphorbia antiqoram*, Cytotoxicity, Medicinal plants.

INTRODUCTION

Cancer has become a leading cause of death in the world because changed disease spectrum by social and environmental risk factors. Cancer is a series of malignant disease with multiple pathological stages (e.g. cancer initiation, promotion and progression) and involved in multiple factors (genetic, environmental, biological, chemical, physical and psychological factors). Cancer is characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth of tumor, proliferate throughout the body and initiate abnormal growth at other sites[1]. Chemotherapy is one of the important treatment methods, but the side effects are difficult to tolerate, and as a result, people are paying more attention in searching for new antitumor agents from natural products[2]. The use of alternative medicines, especially when used in conjunction with conventional cancer treatments, can serve to mitigate the side effects, enhance the uptake of conventional medicines, and, bolster the immune system to fight the cancer. Since these medicines are primarily extracts of naturally occurring flora, their bioavailability is less likely to induce severe immune responses[3]. *Euphorbia antiquorum* is one of the largest armed trees in Euphorbias, with an average height of 5-7 m, it has been known to attain gigantic proportions if left undisturbed. The odour of its latex is pungent and lingering[4].The absorbent prepared from *Euphorbia antiquorum* L is an effective absorbent for the removal of coloring matter present in the dyeing industry effluent[5]. Previous studies in our laboratory has shown that, the latex milk of *Euphorbia antiquorum* can be exploited for its antimicrobial activity and found to be safe to normal cells namely *S. cerevisiae* and chick embryo fibroblast cells[6] The present study was designed to study the cytotoxic effect of methanolic extract of latex milk of *Euphorbia antiquorum*.

MATERIALS AND METHODS

Plant material

The plant specimen was identified and authenticated (Specimen No 365) by Dr.G.V.S Murthy, Scientist E, Director, Botanical survey of India, Tamilnadu. The latex milk was collected from plant by breaking up the stem of *Euphorbia antiquorum*. The latex milk was collected in the morning hours between 7 to 8 a.m in a glass container and maintained in an ice – cold condition till the use of latex for extraction. The collected latex milk was extracted with methanol. The methanolic extract was prepared by dissolving 1.0ml of latex in 5.0 ml of methanol and allowed to evaporate at 60ºC in a in a water bath. The residue was dissolved in minimal volume of DMSO, stored and used for the assays.

Cytotoxicity Screening

Two model organisms were used to screen the cytotoxic activity of *Euphorbia antiquorum.*

1. Brine shrimp lethality bioassay

Artemia salina the brine shrimp is an invertebrate component of the fauna of saline aquatic and marine ecosystem[7]. It plays an important role in the energy flow of the food chain. And it can be used in a laboratory bioassay in order to determine the toxicity. Different concentrations of the latex milk were added and after 24 hours exposure, the live napulii were counted and LC_{50} value was calculated using probit analysis.

2. Primary cell culture

Spleenocytes were used as a source of primary cells. Spleenocytes were isolated from three to four weeks old mice and cultured in DMEM with 10% fetal calf serum.

Parameters analyzed using spleenocytes

The cell proliferation capacity was assessed by MTT assay. The viability of the cells in the presence and absence of etoposide, with or without latex milk at different concentrations and for different time periods was examined by MTT assay as proposed by Igarashi and Miyazawa (2001)[8] The SRB assay provides rapid and sensitive methods for measuring the cytotoxicity and metabolic activity of the cells. The assay was done in the presence and absence of standard chemotherapeutic drug etoposide with or without latex milk proposed by Skehan *et al* [9]. The extent of viability in the treatment group based on the uptake of neutral red dye by viable cells which was then measured photometrically by neutral red assay[10].

RESULTS AND DISCUSSION

Brine shrimp lethality test

The results of the brine shrimp lethality after 24 hrs of exposure to the sample were determined. A chart was plotted using the % of lethality in Y-axis and concentrations of the latex milk extract in X-axis. At lower concentration, the percentage of lethality rate of the shrimps was found to be less which indicates that at low concentration the survival rate increased and protected the effects of test sample. The calculated LC_{50} value for the tested sample was 20µg.

The *n*-hexane fraction of *Myrsine africana* L. showed good cytotoxicity (66.66%) at 1000 µg/ml, while low activity (33.33 and 13.33%) was observed at 100 and 10 µg/ml, respectively. The LD_{50} value calculated was 30.344 µg/ml for *n*-hexane fraction. The chloroform ($CHCl_3$) extract showed low activity at all concentrations 30.0, 16.66 and 13.33% at 1000, 100 and 10 µg/ml respectively. The ethyl acetate (EtAc) fraction showed low brine shrimp lethality at respective test concentrations that was: 26.66, 20.00 and 13.33% at 1000, 100 and 10 µg/ml, respectively[11]. These findings are in agreement with our results and the LC_{50} was found to be 20µg/ml

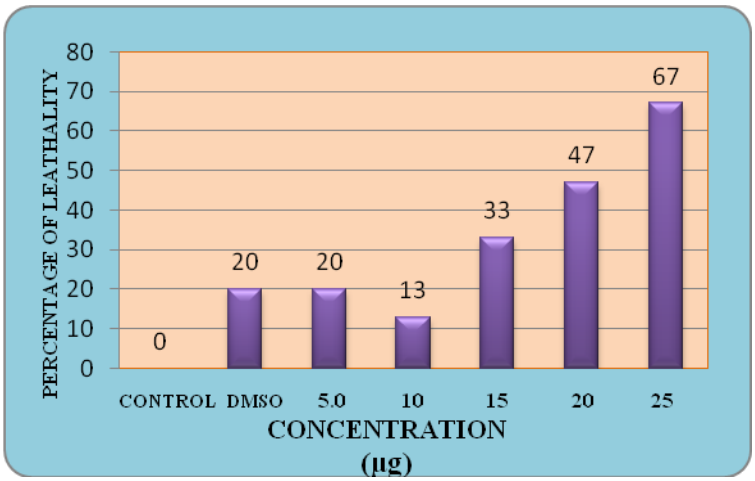

Fig. 1: Brine shrimp lethality test (BSLT)

Cytotoxicity Testing

The percentage of viability of spleenocytes was assessed using different concentrations of plant extracts and also at different time intervals namely 12, 24, 36 and 48 hrs. We checked the cytotoxicity in a time and dose dependent manner. The 24 hours incubation time showed maximum percentage of cell survival and LC_{50} value of methanolic extract of latex milk was found to be 20 µg at different time intervals using probit analysis in SPSS statistical package.

Studies reported by other workers have shown that the potency of methanolic extract of *Euphorbia hirta* calculated in terms of percent decrease in viable Hep-2 cells as compared to the control value. The extract showed dose dependent antitumor activity. The MTT assay showed an anti proliferative activity (IC_{50}) at 625µg/ml of crude extract[12]. These studies support our findings. Cytotoxicity was also tested in the presence of standard chemotherapeutic drug etoposide.

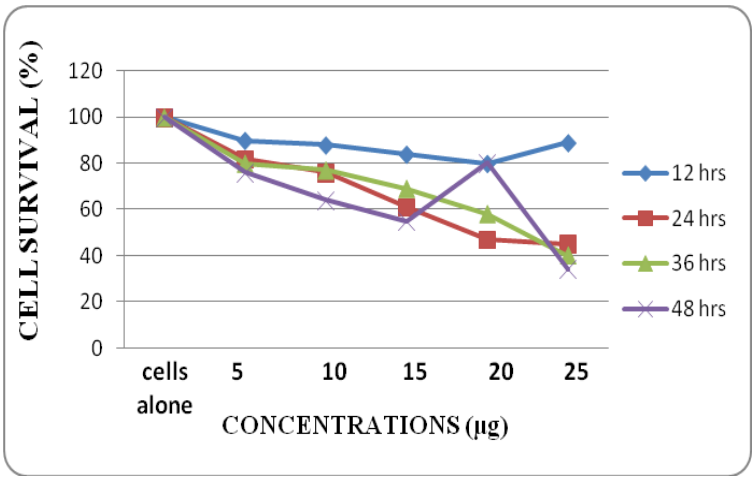

Fig. 2: Dose – Dependent effect of *Euphorbia antiquorum* latex milk on proliferation of spleenocytes by MTT assay

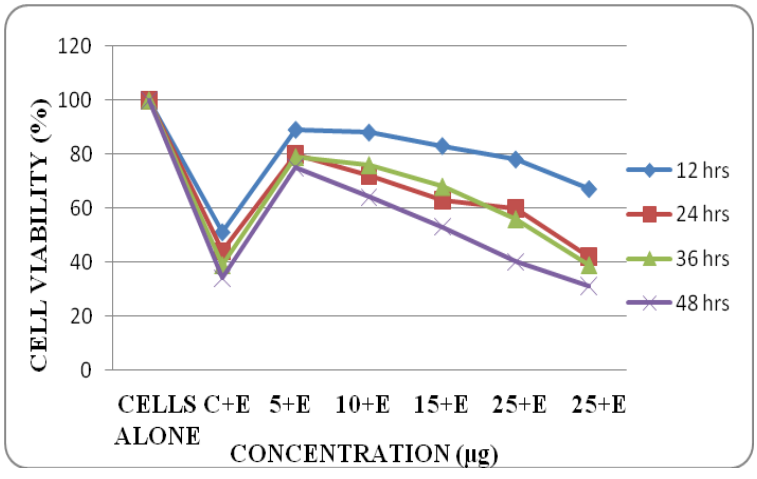

Fig. 3: Dose – Dependent effect of *Euphorbia antiquorum* latex milk on proliferation of spleenocytes co treated with etoposide by MTT assay. E = Etoposide

SRB Assay

The results obtained for the effect of etoposide on cytotoxicity and co treatment with latex milk, as determined by the SRB assay, showed that the viability of the spleenocytes decreased in the etoposide treated group. Only a slight reduction in the viability was observed in the group treated with latex milk extract alone, which showed that the latex milk extract by itself was not toxic to normal cells. However an improvement in viability was observed in the spleen cells treated with etoposide as well as the latex milk. This improvement must be due to the protective effect of latex milk extract against etoposide. Several authors have reported their findings in tune with our studies.

The percentage growth inhibition was found to be increasing with increasing concentration of test compounds of *Solanum Nigrum* on HeLa cell line up to 0.0196 mg/ml and the IC_{50} value of *Solanum Nigrum* on HeLa cell and Vero cell were 847.8 and 0.8724, 0.9088 and 0.1017 respectively by SRB assay[13].

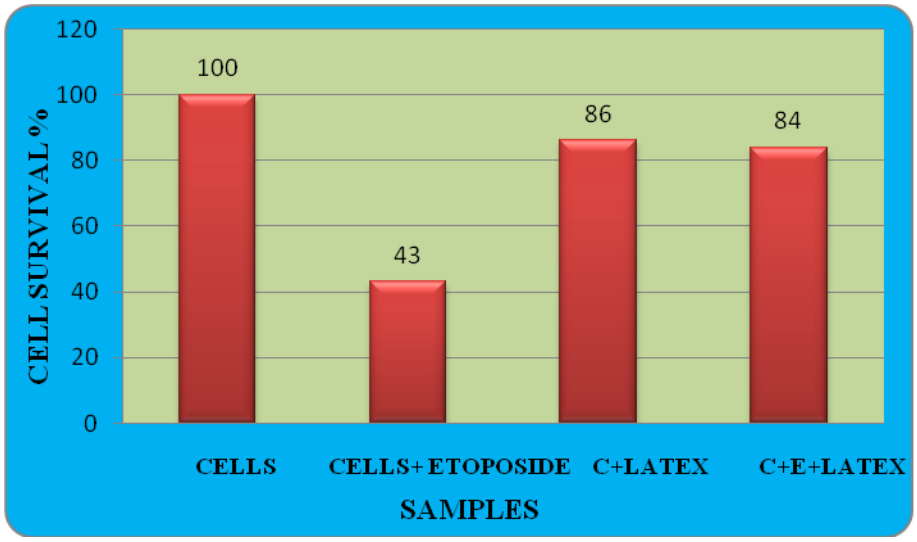

Fig. 4: Effect of latex milk of *Euphorbia antiquorum* linn. on the cell survival of spleenocytes treated with etoposide by SRB assay

The values are mean of duplicates. The control was fixed as having 100% cell survival and the percent survival in the other groups was calculated.

Neutral Red Assay

Neutral red assay, which is based on the uptake and accumulation of the supravital dye neutral red by viable cells. The result obtained in the neutral red assay showed that etoposide treatment caused a marked decrease in the viability of the cells and the co-administration of latex milk extract increases the cell survival. The extent of cell survival in the latex milk extract alone treated group was comparable to that of control cells suggesting that the latex milk of *Euphorbia antiquorum* was not toxic to the spleen cells. Similar results have been reported that the cytotoxic effects of the crude methanol and fractionated extracts (hexane, ethyl acetate) of *C. mangga* against six human cancer cell lines, namely the hormone-dependent breast cell line (MCF-7), nasopharyngeal epidermoid cell line (KB), lung cell line (A549), cervical cell line (Ca Ski), colon cell lines (HCT 116 and HT-29), and one non-cancer human fibroblast cell line (MRC-5) by neutral red cytotoxicity assay. The crude methanol and fractionated extracts (hexane and ethyl acetate) displayed good cytotoxic effects against MCF-7, KB, A549, Ca Ski and HT- 29 cell lines, but exerted no damage on the MRC-5 line[14].

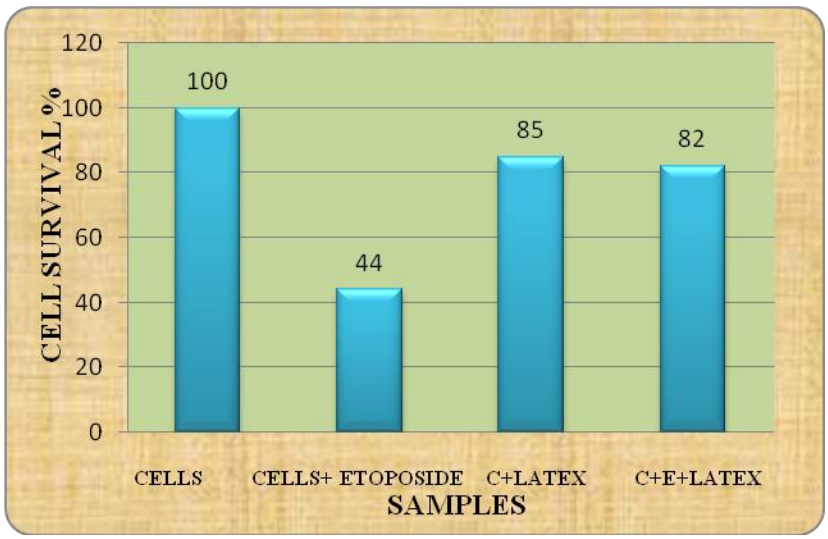

Fig. 5: Effect of latex milk of *Euphorbia antiquorum* on the viability of spleenocytes treated with etoposide as determined by the Neutral red assay

The values are mean of duplicates. The control was fixed as having 100% cell survival and the percent survival in the other groups was calculated relative to this.

CONCLUSION

Methanol extract of latex milk of *Euphorbia antiquorum* was tested for the cytotoxic and apoptotic activity using brine shrimp and mice spleenocytes and it showed latex milk is safe to brine shrimp and normal spleen cells in lower concentration. The need of the present study is to develop drugs that can potentially target cancer cells by means of their inherent difference to normal cells. The development of such drugs with differential action will be very valuable in cancer chemotherapy without the observed side effects. These findings potentiate the use of latex milk of *Euphorbia antiquorum* in combination therapy when this extract can overcome the toxic side effect of chemotherapeutic drug, because it is safe to normal cells. If it's anticancer activity is probed further using cancer cell lines we can see if it exhibits a differential response to normal cells and cancer cells. The potential use of *Euphorbia antiquorum* as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude latex milk extract and the judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumor in individuals who are highly susceptible to developing a tumor.

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