# Evaluation of Anticancer Properties of *Acalypha alnifolia* Klein ex Willd - *In vivo*

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***Abstract*----**Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Therefore an attempt has been made to review different in vitro and in vivo methods for estimating anticancer properties of natural products from medicinal plants. In the present study, we investigated the in vivo antitumor effects of crude extract of *Acalypha alnifolia* Klein ex Willd on mice with DLA cell suspension. The extract showed a significant increase in the life span and a decrease in cancer cell number, tumor weight and tumor volume. The protective effect of the extract on the hemopoietic system at the dose level 250 and 500 mg/kg were noted. From the result it can be found that the methanol extract of *Acalypha alnifolia* showed significant ($p<0.001$) in vivo cytotoxic effect when compared to the tumor control group.

***Keywords*---***Acalypha alnifolia* Klein ex Willd, DLA cell line, *In vivo* anticancer, methanol extract

## I. Introduction

Herbs have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [Dahanukar *et al*., 2000], [Exarchou *et al*., 2002]. Medicinal plants are an important resource to traditional society's health care systems. In today's world the percentage of people using chemicals drugs increases with their side effects. Many cancer patients have received chemotherapeutic agents during the course of illness, treatment based on cell destruction potential by growth interruption. Within this group, plants have been thoroughly used in the treatment of these maligned diseases. Many drugs have been extracted from plants. It is worth to mention as example the use of vinblastine, vincristine, podophyllotoxin, taxol. The latter is a potent anticancer agent, which was extracted from species of the genus Taxus, constituting the most used antineoplastic agent at the present time [Guillermina *et al*., 2006].

Cancer is a fatal disease which spreads around the world in a fast track. Now a days, drugs and UV radiations are being used to treat cancer, but these have side effects, which create fatal effects. The life saving solution for this problem can only be dealt with the Ancient herbicology [Shivayogi *et al*., 1999].

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Natural products have been the starting point for the discovery of many important search for pharmacologically important substance from plant source. The plant *Acalypha* belongs to the sole genus subtribe acalyphinae of the family Euphorbiaceae which comprises about 570 species, a large potion of which are ornamental plants. Some of the species are well known in folk medicine and a few have actually appeared in homeopathic pharmacopoeia. *A.alnifolia* is showing various biological and pharmacological activities. Many species of Euphorbiaceae are used because of their medicinal, toxic or economically interesting properties. Some of the species are well known in traditional medicine and a few have actually appeared in the Homeopathic pharmacoploeia of [United States, 1941] and [India, 1971].

The literature survey revealed that there are no scientific studies carried out regarding anticancer properties of the leaves of *A.alnifolia*. The main objective of this study is to identify a good candidate phytochemical and for combating cancer. In the wake of identifying natural sources rich in antioxidant and anticancer, *Acalyha* species is believed to contain many medicinal properties and antioxidant and anticancer potentials. Hence the plant, *A.alnifolia* has been chosen for the present study to evaluate its *in vitro* and *in vivo* anticancer properties of leaf extracts.

## II. Materials and Methods

### Plant Collection and Identification:

*A.alnifolia* used in the study was identified in the Botanical Survey of India-Southern Circle-Coimbatore, Tamil Nadu, India. The reference material has been kept under the reference: [No: BSI/SRC/5/23/2010-11/Tech-1506]. Fresh plant parts were collected randomly from the region of Shervarayan Hills, Yercaud, Salem District, Tamil Nadu. Leaves were taken for investigation of anticancer properties. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

### Mice and Tumour System

Female Swiss albino mice (20±2 g, 6–8 weeks old) were used for acute toxicity and anticancer study. Mice were housed in open top cages and maintained on food and water *ad libitum*. Room temperature was maintained at 22±2°C with the light and dark cycle of 14/10 hrs. Dalton's lymphoma [DLA] is maintained in ascetic form by serial transplantation in Swiss albino mice or *in vitro* cell culture system by serial passage. Irrespective of whether the cells are obtained from *in vitro* culture or from ascetic fluid they exhibited typical phenotypic features.

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**Preparation of Suspensions and Solutions [**Santosh kumar *et al*., 2007]

The test drugs and standard 5-Fluorouracil (5-FU, procured from Ranbaxy Laboratories, New Delhi) were suspended in distilled water using sodium carboxy methyl cellulose (0.3%) and administered orally to the animals with the help of an intra gastric catheter.

**Evaluation of Antitumor Activity of Test Drugs in Dalton's Lymphoma-Bearing Mice Model**

The animals, total of 42 were divided into seven groups (six mice in each group) Group 1- Group 5. Under sterile condition, about 0.5 ml of DLA cell suspension ($2\times10^6$cells/ml) was inoculated intraperitoneally to each mouse of Group 2 to Group 5 at day zero. After two days tumor inoculation the animals were treated as fallows,

**Group 1**: **Normal control** and received sodium CMC suspension (0.3%).

**Group 2**: **Tumour control** and received sodium CMC suspension (0.3%).

**Group 3**: **Positive control** was treated with -fluorouracil (3.4 mg/kg, p.o).

**Group 4**: Treated with **LM (200 mg/kg, p.o)** in sodium CMC suspension

**Group 5**: Treated with **LA (200 mg/kg, p.o)** in sodium CMC suspension

The treatment was continued for the next 10 days. On day $11^{th}$ day, i.e. after 24 hrs fasting after last dose, blood samples were collected from the animals by retro-orbital puncture under mild anesthesia (diethyl ether) and were subjected to hematological parameters and animals were kept to check the parameters, average life span, percentage increase in life span (% ILS), body weight analysis, packed cell volume, viable tumor cell count and hematological parameters.

## III. Results and Discussion

***In vivo* Anticancer Activity**

The results showed that the methanolic extract of leaf exhibited the most activity against ascites tumour cell. A good example of such Asian foods are Indian food ingredients which can be used in preventive strategies aimed at reducing the incidence and mortality of different type of cancers because of their antioxidative [Devasagayam and Sainis, 2002], antimutagenic and anticarcinogenic properties [Arora *et al*., 2003]. Therefore, it is concluded that the methanolic extract of leaf had selective toxicity against cancer cells with little damage to normal cells, indicating suitability of the plant for cancer treatment [Halliwell and Gutteridge, 1998].

The petroleum ether and ethanol extracts of *Acalypha indica* at 600mg/kg body weight of female albino rats, showed estrogenic activity and it was effective in causing anti-implantation activity [Weber *et al*., 1996].

The animals of tumour control group survived for a period of 22.00 ± 0.25 days. The treatment with different dose showed dose dependant increase in the average life span of DLA bearing mice, when compared to tumor control group. Treatment with methanolic extract increased the life span significantly with percentage increase ranging from 22.32 to 50.61. The standard drug, 5-FU at 3.4mg/ kg b.wt, significantly increased to life span to 42.50 ± 0.50 days, i.e. 95.49 per cent increase in the average life span of tumor bearing mice (Table 1).

The increase in body weight of DLA bearing mice was found to be 42.60 ± 0.50%. Treatment with test drugs showed a significant and dose dependant reduction in percentage increase in body weight ($p<0.001$), when compared to tumor control group. However among the test drug, methanolic extract showed better

TABLE-1: EFFECT OF METHANOLIC EXTRACT ON ANTITUMOR PARAMETERS OF DLA BEARING MICE

| Parameters | Normal | DLA control ($1 x10^6$ cells/ml/ mice) | DLA + 5-FU | DLA + Methanolic extract (250 mg/kg) | DLA + Methanolic extract (500 mg/kg) |
|---|---|---|---|---|---|
| Average life span, Days | --- | 22.00 ± 0.25 | 42.50 ± 0.50$^{f}$ | 27.36 ± 0.58$^{f}$ | 33.45 ± .85$^{f}$ |
| Increase in life span, % | --- | --- | 95.49 | 22.32 | 50.61 |
| Increase in body wt, % | --- | 42.60 ± 0.50 | 28.46 ± 0.76$^{f}$ | 36.76 ± 2.76$^{f}$ | 31.36 ± 1.45$^{f}$ |
| Packed cell volume, ml | --- | 8.62 ± 0.05 | 1.04 ± 0.01$^{e}$ | 5.13 ± 0.01$^{e}$ | 2.84 ± 0.01$^{f}$ |
| Viable tumour cell count (x $10^7$ cells/ml) | --- | 567.00 ±10.75 | 318.00 ± 12.21$^{f}$ | 468.00 ± 5.78$^{d}$ | 338.63 ± 10.65$^{f}$ |
| Total WBC ($10^3$/mm$^3$) | 8.78 ± 0.50 | 18.75 ± 0.43$^{c}$ | 9.95 ± 1.34$^{f}$ | 14.63 ± 0.78$^{e}$ | 11.16 ± 0.60$^{f}$ |
| RBC ($1x10^6$/mm$^3$) | 11.38 ±0.65 | 6.98 ± 0.71$^{c}$ | 10.85 ± 0.56$^{f}$ | 7.85 ± 0.36$^{f}$ | 9.30 ± 0.10$^{f}$ |
| Hgb (g/dl) | 13.42 ±0.38 | 6.96 ± 0.58$^{b}$ | 12.56 ± 0.85$^{d}$ | 8.75 ± 1.50 | 11.85 ± 1.85 |

* Values are expressed as mean ± STDEV for six animals in each group $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ between normal and tumor group values. $^{d}P<0.05$, $^{e}P<0.01$ and $^{f}P<0.001$ between tumor control and treated groups

reduction in percentage increase in body weight. The standard drug, 5-FU was found to be more potent in inhibits the increase the body weight of animal with only 42.60 ± 0.50% ($p<0.001$). Treatment with test drug reduced the packed cell volume of ascetic fluid when compared with tumor control group. Among test drugs, methanolic extract at the dose of 250mg/kg b.wt significantly reduced the packed cell volume ($p<0.001$). Similar trend was observed in viable tumor cell count also. Standard drug 5-FU showed significant reduction in packed cell volume and viable tumor cell count ($p<0.001$).

Inoculation of DLA cells resulted in significant increase in the levels of total WBC ($p<0.001$) and a significant reduction in the levels of RBC ($p<0.001$), when compared to normal animals. Treatment with test drugs showed dose dependant reversal of these changes towards normal values. Among the methanolic extract, treatment was found to be more potent in normalizing RBC and WBC levels. Standard drug, 5-FU exhibited potent activity by restoring the levels of hematological changes towards normal ($p<0.001$).

## IV. Conclusion

The findings of the present study with DLA tumour cell suggest that the methanolic leaf extract possessed most anticancer activity. This study is a positive demonstration of the utility of screening South Indian endemic Euphorbiceae plant for their food and medicinal uses and this is the first report of the anticancer analysis of *A.alnifolia*. The presence of active compounds in *A.alnifolia* will allow for the preparation of new drug with new biological agents as a result of obtaining that active agent from plants through different methods and purification process.

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The awards she had won so far are 1) "Received Best poster award" for CANDATA: A Data base of medicinal plants used for the treatment of cancer. National seminar on scientific validation of medicinal plants titled on 'Herbal Focus – 09' conducted by Srimad Andavan arts and science college conducted on January 24th and 25th, 2010; 2) "Best Paper award" for Antioxidant activity in *Acalypha alnifolia* in national symposium on Innovations and Advances in Science and Technology Conducted by Indian Science Congress Association on Feb 27, 2014 in Pondicherry; 3) "Fellowship award" in Bose science society, Pudukkottai. 4) "HAR GOBIND KHORANA BEST YOUNG INVESTIGATOR AWARD 2015 FOR BIOTECHNOLOGY" Received by Bose science society, Pudukkottai. 5)"Achiever Award" is received for the excellence in the field of science given by A.S Media Vision, on 11th June, Chennai.

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