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Research Article

# ANTITUMOR ACTIVITY OF METHANOLIC EXTRACT OF *TERMINALIA CATAPPA* LEAVES AGAINST EHRLICH ASCITES INDUCED CARCINOMA IN MICE

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**ABSTRACT**

*Terminalia catappa* belongs to the family combretaceae and is popularly known as badam.It is a well known herb in Ayurvedic system of medicine The aim of the present study is to evaluate the effect of methanolic extract of leaves of *Terminalia catappa* against Ehrlich ascetic Lymphoma (ELA) in Swiss albino mice.The tumor was induced in mice by intraperitoneal injection of ELA cells (1X106cells/mice). Methanolic extract of *Terminalia catappa* was administered to the experimental animals at a dose of 75µg/kg/day after 24 h of tumor inoculation. The antitumor effect of extract was evaluated by assessing in vitro cytotoxicity, increase in lifespan, hematological parameters and liver enzymes. The methanolic extract brought back the altered levels of the hematological parameters and liver enzymes. Thus the present study revealed that methanolic extract of *Terminalia catappa* possessed significant antitumor activity.

**Key words**: ELA, *Terminalia catappa*, liver enzymes, Cytotoxicity, WBC

## INTRODUCTION

*Terminalia catappa* L. is a Combretaceous plant whose leaves are widely used as a folk medicine in Southeast Asia for the treatment of dermatitis and hepatitis[1]. The crude methanolic extract possessed prominent antibacterial activity[2]. The methanolic extract of the leaves were also found to possess antimicrobial activity[3]. More and more pharmacological studies have reported that the extract of *T. catappa* leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase,anti-inflammatory, antidiabetic effects and hepatoprotective anti-inflammatory activity [4-7]. The extract of *T.catappa* leaves inhibit Lewis lung carcinoma cells that contribute to lung cancer[8]. Previous studies from our research group reported that *Terminalia catappa* leaf protein has antioxidant activity against ELA implanted Swiss albino mice[9]. The objective of the present study is to evaluate the antitumor activity of methanolic extract of *Terminalia catappa* in ELA induced Swiss albino mice.

## MATERIALS AND METHODS

### Plant Material

Fresh leaves of *Terminalia catappa* were collected in area free of pesticides and other contaminants from the area surrounding of Coimbatore Dt, Tamilnadu.The collected leaves were washed thoroughly and blotted dry with filter paper and used for the preparation of the extract.

### Preparation of Methanol Extract

The extraction was undertaken with 20 g of powdered plant material and 200ml. of light petroleum ether (b.p. $40^0 - 60^0$ C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll and lipid de waxing. The treated material was dried and extracted with methanol using Soxhlet apparatus for 4 hours.The extract was concentrated in vacuo using a rotary evaporator.

### Tumor Cell lines

Ehrlich ascites carcinoma (EAC) cells were obtained under the courtesy of Amala Cancer Research Center, Thrissur, India. They were maintained by weekly intra-peritoneal inoculation of $10^6$ cells/per mouse.

### Animals

In bred Swiss albino mice weighing on an average 20-25 g procured from Small Animal Breeding Station, Medical College, Perundurai and Tamilnadu were used to evaluate the antitumorigenic effect in ELA tumor induced Swiss albino mice. These animals were maintained for two weeks under environmentally controlled conditions with free access to standard food (Lipton, India) and water ad libitum, prior to the experiments. All animal experiments were carried out according to the guidelines prescribed by Animal Welfare Board and with the approval of Animal Ethic Committee (Register no: 623/02/b/CPCSEA). The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation of 1 x $10^6$ cells in 100 µl of PBS. After 10-15 days, the cells were drawn from the intraperitoneal cavity and used for the in vitro studies.

### Determination of in vitro cytotoxic activity using EAC cell lines

*In vitro* cytotoxic studies were carried out to find out the 50 per cent effective concentration ($EC_{50}$) of methanolic extract of *Terminalia catappa* by trypan blue exclusion method[10]. The number of (stained) dead cells randomly in every 200 cells was counted[11]. The results were recorded as percent protection against tumor growth calculated as the difference between the number of dead cells in treated and untreated animals expressed as a percentage of the number of dead cells in untreated (control) animals.

### Antitumor activity

Male Swiss albino mice were divided into 3 groups (n = 6). All the groups were injected with EAC cells $1X10^6$ cells/mouse) intraperitoneally except Group I. This was taken as day Zero.

Group I - Normal control.

Group II - Disease Control, EAC cell line ($1x10^6$ cells /mouse).

Group III - EAC cell line (1x106 cells/mice) treated with 75µg of extract in 100µl of Dimethyl sulphoxide (DMSO) /kg.bw

All this treatments were given 24 h after the tumor inoculation, once daily for 14 days. On day 14, the Increase in life span (ILS) was determined. After the last dose and 24 h fasting, six mice from each group were sacrifice.The blood was collected from the animals by retro-orbital puncher under slight anesthesia (diethyl ether) conditions; and the hematological parameter such as hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count[12] and the remaining blood was centrifuged and serum was used for the estimation of liver enzymes like ALT[13], AST[13] and ALP[14].

### Statistical Methods

All values are expressed as mean± SD

## RESULTS AND DISCUSSION

Ehrlich tumor was initially described as spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascetic form it has been used as a transplantable tumor model to investigate the antitumor effect of several substances[15]. The methanolic leaf extract of *T.catappa was* found to be cytotoxic towards Ehrlich ascites carcinoma cells only at higher concentration

Table 1). At concentration of 75 µg and 100 µg produced 100% cell death. The methanolic leaf extract produced a concentration dependent cytotoxic effect to EAC cells.

The results of the present study clearly demonstrate the tumor inhibitory activity of against *T. catappa* extract EAC. The reliable criteria for evaluating an anticancer drug are prolongation of lifespan of the animal and decrease in WBC count of blood. Our results have shown an increase in lifespan accompanied by a reduction in WBC count in T. *catappa* extract treated mice. It had significant effect on increasing the life span of ascities tumor bearing animals and also found to reduce the viable EAC cells in animal models. These results clearly demonstrate the antitumor effect of *T. catappa* extract against EAC. In cancer chemotherapy the major problems are of myelosuppression and anemia.[16] The anemia encountered in tumor bearing mice is mainly due to reduction in RBC and hemoglobin and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions.[17]Treatment with *T. catappa* extract restored the hemoglobin content, RBC and WBC cell count to normal values. This indicates that *T. catappa* extract possesses protective effect on the haematopoietic system.

It was reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the liver. The significantly elevated levels of AST, ALT and ALP in serum of tumor-inoculated animals indicated liver damage and loss of functional integrity of cell membranes[18]. The significant reversal of these changes towards the normal by *T. catappa* extract treatment in most of the cases demonstrated the potent hepatoprotective and antioxidant nature of *T. catappa* extract. The antioxidant nature of *T. catappa* extract was also evident by the in vitro studies. Preliminary phytochemical analysis of this extract showed the presence of phenolic compounds, flavonoids and tannins.Plants with high total phenol content are known to possess strong antioxidant properties[19]. Reduction in the levels of these towards the respective normal values in liver is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by tumor inoculation.

## CONCLUSION

The methanol extract of *T. catappa* was effective in inhibiting the tumor growth in ascitic tumor model. The biochemical and hematological studies supported its antioxidant and hepatoprotective properties. The plant merits further investigation in an ascitic model at low doses and to elucidate its mechanism of action and isolation of its active constituents.

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**Table 1: Effect of methanolic extract of *T.catappa on* cytotoxity of Ehrlich ascites carcinoma**

| S.no | Concentration /ml | % of cytotoxicity EAC cells |
|---|---|---|
| 1 | Control | 8 |
| 2 | 10 | 28 |
| 3 | 25 | 46 |
| 4. | 50 | 75 |
| 5 | 75 | 100 |
| 6 | 100 | 100 |

**Table 2: Effect of *T. catappa* extract on the life span**

| Treatment | Number of animals with tumour | Increase in life span (%) |
|---|---|---|
| Normal control | 0/6 | 100% |
| ELA control | 6/6 | 42% |
| ELA+*T.catappa* extract | 6/6 | 90% |

**Table 3. Effect of methanolic extract of *T. catappa on* the hematological parameters.**

| Groups | Hemoglobin (%) | RBC $1x10^6$ cells/ $mm^3$ | WBC $1x10^3$ cells/ $mm^3$ |
|---|---|---|---|
| Group I Normal control | 12.4±0.289 | 5.35±0.361 | 10.91±0.43 |
| Group II Disease control | 8.63±0.216 | 3.28±0.194 | 20.11±0.729 |
| Group III ELA+Terminalia extract | 9.81±0.285 | 4.516±0.331 | 17.01±0.489 |

The values are the mean ±SD of six animals

**Table.4 Effect of *T.catappa* methanolic extract on serum AST, ALT and ALP**

| Groups | AST(U/ L)[a] | ALT(U/ L)[a] | ALP (U / L)[b] |
|---|---|---|---|
| Group I Normal control | 48.76± 0.585 | 92.83 ± 3.12 | 125.83 ± 3.48 |
| Group II Disease control | 84.55 ± 1.42 | 98.58 ± 3.07 | 195.66 ± 3.44 |
| Group III ELA+Terminalia extract | 53.33 ± 0.98 | 94.5 ± 2.25 | 135.0 ± 4.47 |

The values are the mean ± SD of six animals
a -micromole of pyruvate formed / minute
b- micromole of phenol formed / minute

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