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Evaluation of anticancer potential of *Terminalia chebula* Fruits against Ehrlich Ascites Carcinoma induced cancer in mice

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Abstract

This study was designed to determine the in vivo and in vitro anticancer potential of the ethanolic extract of *Terminalia chebula* (ETC) fruits against Ehrlich Ascites Carcinoma (EAC) induced cancer in swiss albino mice. The anticancer activity was assessed using in vitro cytotoxicity, mean survival time, tumor volume and hematological studies. The reliable criteria for evaluating the potential of any anticancer agent is the prolongation of lifespan of the animal and decrease in WBC count of blood.The high dose of ETC (200 mg/kg, orally) significantly reduced the tumor growth which was demonstrated by increased lifespan of the mice and restoration of hematological parameters. ETC was also found to be cytotoxic in the in vitro parameter which shows that ETC possesses significant anticancer potential.

Keywords: Cancer, Ehrlich Ascites Carcinoma (ETC), *Terminalia chebula* (TC), Cytotoxicity, Flavonoids

Introduction

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize. Most cancers form a tumor but some, like leukemia, do not. Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age.[1] Cancer causes about 13% of all human deaths.[2] According to the American Cancer Society, around 7.6 million people die every year from cancer.[3]

The World Health Organization (WHO) has estimated that approximately 80% of the world's population depends on traditional medicines for meeting their primary health care needs due to the problems that exist with the current chemotherapeutic regimens. Most of these traditional medicines are derived from plants as they have interesting biological activities with potential therapeutic applications.[3] *Terminalia chebula* (TC), belonging to family Combretaceae and commonly known as "Black Myrobalan" is found in India as well as in many Asian countries. *Terminalia chebula* is called the

"king of medicines" and is always listed first in the Ayurvedic meteria medica because of its extraordinary powers of healing. In Ayurveda it is considered that TC has capacity to destroy all diseases and eliminate all wastes from the body and also it is known to promote tissue growth and health. *Terminalia chebula* is traditionally used in formulation for anti-diabetic, anti-inflammatory, laxative, antibacterial, antifungal, cardiotonic, diuretic, hyperlipidemic activity and jaundice.[4, 5] The plant is also having anthelmentic, aphrodisiac and restorative properties.. Except this, fruit of the plant has a wide antibacterial and antifungal spectrum and also inhibits growth of *E. coli*, the most common organism responsible for urinary tract infection.[4, 6] The major bio-active constituents of the fruit include tannins, anthraquinones, chebulinic acid, chebulagic acid, chebulic acid, ellagic acid and gallic acid. The other minor compounds include polyphenolic compounds, triterpene glycosides, terchebulin, punicalagin, terflavin-A, flavonoids like rutin and quercitin, terpenene glycosides, arjungenin and arjunglucoside-I and a small quantity of phosphoric, succinic, syringic and quinic acids.[6, 7]

Although, many benefits of *Terminalia chebula* have been claimed but only few of them are scientifically authenticated. Hence, we undertook the present study to evaluate the in-vivo and in-vitro antitumour activity of *Terminalia chebula* extract against EAC (Ehrlich Ascites Carcinoma) in mice.

Materials and Methods

Plant extract

The fruits of *Terminalia chebula* were collected and authenticated from the Botanical Garden, Surat, India and a voucher specimen was deposited (No. AA-33/12). The fruits were air dried, pulverized and were extracted with 90% ethanol (1 L) by refluxing for 24 hrs and then, cooled. The residue was removed by filtration and discarded. The filtrate obtained was evaporated to dryness and the last traces of the solvent present were removed under reduced pressure using rotary evaporator. This dried extract (78 gm) was resuspended in distilled water and was further used for pharmacological studies.

Cancer cell lines

Ehrlich Ascites Carcinoma (EAC) cells were obtained through the courtesy of National Facility for Animal Tissue and Cell Culture, Pune, India. They were maintained by weekly intra-peritoneal inoculation of 106 cells/mouse.

Animals

Male adult Swiss Albino mice (25-30 g) were used. The animals were housed in well ventilated room in a controlled environment (temperature 25±2ºC and 12 h dark/light cycle) with standard laboratory diet and water ad libitum. Experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (Reg. No. 10/2010/CPCSEA).

Short term toxicity studies

Ehrlich Ascites Carcinoma (EAC) cells were aspirated from the peritoneal cavity of mice and washed three times in Hank's Buffered Salt Solution (HBSS).[8] One million cells were incubated with different concentrations of the extract (10-200 µg/ml) in a total volume of 1.0 ml for 3 h at 37ºC. The viability of cells was then determined using tryptan blue exclusion method.[9]

Determination of Mean Survival Time (MST)

Animals were inoculated with 1x106 cells per mice on day Zero and treated after 24 h of inoculation with ethanolic extract of *Terminalia chebula* (ETC) at a dose of 100 mg/kg and 200 mg/kg orally. The control group was treated with same volume of normal saline solution. All treatments were continued for 10 days. The MST of each group consisting of 8 mice was noted and the antitumor efficacy of ETC was compared with that of 5-fluorouracil

(5-FU, 20 mg/kg/day, i.p. for 10 days).[10] The MST of the treated groups was compared with that of the control group using the following calculation:

$$\text{Increase in lifespan} = \frac{T - C}{C} \times 100$$

Where, T = number of days the treated animals survived.

C = number of days control animals survived.

Determination of Hematological Parameters

In order to determine the effect of ETC on the hematological parameters of EAC bearing mice, comparison was made amongst four groups (n = 8) of mice on the 14th day after transplantation. The four groups comprised of (1) normal mice, (2) tumor bearing mice treated with normal saline (control) and (3 and 4) tumor bearing mice treated with ETC (100 mg/kg and 200 mg/kg body weight respectively). Blood was drawn from each mouse from tail vein under sterilized conditions and the red blood cell (RBC) count, hemoglobin content (Hb) and white blood cell (WBC) count were determined.[10, 11]

Volume of Tumor mass

Mice were divided into three groups (n=8). Tumor cells (1 X 106 cells/mice) were injected into the right hind limb (thigh) of all the animals intramuscularly. The mice of Group 1 were tumor control. Group 2 and group 3 received ETC (100 mg/kg and 200 mg/kg) orally for 5 alternate days. The dose was selected based on toxicity studies which showed no toxicity up to 3 g/kg (orally). Tumor mass was measured from the 11th day of tumor induction.[12-14] The measurement was carried out every 5th day for a period of 30 days. The volume of tumor mass was calculated using the formula,

$V = 4/3\pi r2$ where, r is the mean of r1 and r2 which are two independent radii of the tumor mass.

Results

Short term toxicity studies

ETC produced a concentration dependent anticancer activity against Ehrlisch Ascites Carcinoma cells i.e. it was found to be significantly viable at higher doses i.e. 100 µg and 200 µg (Table 1).

Table 1: Effect of ETC on cytotoxity of EAC cell lines

Concentration/ml	% viability in EAC cells
Normal saline	6
10 µg	14
25 µg	29
50 µg	49
100 µg	86
200 µg	100

ETC: Ethanolic extracts of *Terminalia Chebula* fruits

EAC: Ehrlich Ascites Carcinoma

Determination of Mean Survival Time (MST)

The MST for the control group was found to be 27 days, while it was 39±1.97 days for ETC (100 mg/kg orally), 47±1.12 days for ETC (200 mg/kg/day orally) and 51±1.23 days for positive control group treated with 5-fluorouracil (20 mg/ kg

i.p. per day) respectively. The increase in the lifespan of tumor bearing mice treated with ETC (100 and 200 mg/kg) and 5fu was found to be 44.44%, ($p < 0.05$), 74.07% ($p < 0.05$) and 88.88% ($p < 0.001$), respectively as compared to the control group (Table 2).

Table 2: Effect of ETC on MST of tumor bearing mice

Treatment	Mean survival time (day)	% increase in lifespan
Control	27±1.08	-
5-FU (20mg/kg/day, i.p.)	51±1.23*	88.88
ETC (100 mg/kg, orally)	39±1.97**	44.44
ETC (200 mg/kg, orally)	47±1.12**	74.07

*$p < 0.05$ and **$p < 0.001$ compared with control group (n=8 animals in each group)

5-FU = 5- fluorouracil. Values are expressed as mean ± SEM.

ETC: Ethanolic extracts of *Terminalia Chebula* fruits; MST: Mean survival time

Determination of Hematological Parameters

Hematological parameters of tumor bearing mice on the 15th day showed significant changes when compared with the normal mice (Table 3). The control group showed fall in Hb level. Treatment with ETC was able to change these altered parameters to nearly normal values.

Table 3: Effect of ETC on hematological parameters

Treatment	Hb (g %)	RBC (10^6 cells/mm^3)	WBC (10^3 cells/mm^3)
Normal mice	14.2±0.1	4.6±0.23	7.9±0.15
Tumor bearing mice (control)	7.2±0.26	2.1±0.37	27.5±1.2
Tumor bearing mice (ETC 100 mg/kg, orally)	9.3±0.31*	3.2±0.75*	18.6±0.98*
Tumor bearing mice (ETC 200 mg/kg, orally)	13.7±0.01**	4.2±0.67**	11.1±1.12**

*$p < 0.05$ and **$p < 0.001$ compared with control (n=8 animals in each group)

Values are expressed as mean ± SEM. ETC: Ethanolic extracts of *Terminalia Chebula* fruits

Volume of Tumor mass

The result showed that there was significant reduction in tumor volume in EAC treated animals. Significant difference was seen in the both low dose and high dose treated group from 5th and 10th day onwards respectively (Table 4).

Table 4: Effect of ETC on volume of tumor mass

Treatment	Tumor volume mm^3					
	5^{th} day	10^{th} day	15^{th} day	20^{th} day	25^{th} day	30^{th} day
Tumor bearing mice (control)	0.22±0.01	0.41±0.01	0.55±0.09	0.79±0.02	0.98±0.07	1.24±0.06
Tumor bearing mice (ETC 100 mg/kg, orally)	0.21±0.04	0.36±0.06*	0.43±0.09*	0.60±0.01*	0.89±0.01*	1.11±0.03*
Tumor bearing mice (ETC 200 mg/kg, orally)	0.18±0.04**	0.22±0.05**	0.26±0.07**	0.31±0.02**	0.32±0.03**	0.35±0.09**

*p < 0.05 and **p < 0.001 compared with control (n=8 animals in each group)

Values are expressed as mean ± SEM. ETC: Ethanolic extracts of *Terminalia Chebula* fruits

Statistical methods

All values are expressed as mean±SEM. The data of all the parameters were statistically analyzed by one-way ANOVA. P values <0.05 were considered significant.

Discussion

Although *Terminalia chebula* has long been used as traditional medicine, very few authentic scientific studies are available. Recent studies have revealed that many constituents from TC have a wide range of biological actions including antidiabetic, hepatoprotective, cardiotonic and antibacterial activities.[6, 7] Some studies have revealed that the fruit of TC is source of flavonoids, tannins, ellagic acid etc. which suggests that TC can be a potential candidate as anticancer agent.

In this study, we investigated the in vivo and in vitro anticancer activity of ETC against EAC cells in swiss albino mice. The reliable criteria for evaluating the potential of any anticancer agent is the prolongation of lifespan of the animal and decrease in WBC count of blood. Decrease in tumor volume and WBC count observed in the present experiment can be considered as important factors for the enhancement of the lifespan of EAC bearing mice. Hertog et al.[15] has suggested that an increase in the lifespan of ascites bearing animals by 25% can be considered as indication of significant anticancer activity of the drug. This observation suggests the effectiveness of ETC against EAC cells in mice as it increased the lifespan by almost 88%. Moreover, ETC was also able to restore the Hb content, RBC & WBC count to near normal values. Thus, counteracting the major problem of myelosuppression and anemia, associated with cancer chemotherapy. This indicates that ETC possesses anticancer activity as well as protective action on hemopoetic system.

Moreover, antitumor activity of flavonoids and ellagic acid isolated from several sources other than TC has been reported.[16] Flavonoids like; rutin and quercetin have been shown to possess antimutagenic and antitumor effects. Also, quercetin has been proved to inhibit human breast cancer cells[17] and prostate cancer cells.[18]

The present observation suggests that flavonoids and several other compounds present in TC extract might be responsible for its cytotoxic and anticancer properties. Identification and characterization of the active principles

from TC extract needs to be done to support this hypothesis. Thus, from this study, it is likely that TC ethanolic extract has high cytotoxic and antitumor properties, suggesting a potential role of TC as a powerful chemotherapeutic agent for cancer. However, further research work is required to establish the exact mechanism of action of ETC at molecular level. This study should help to confirm the effectiveness of *Terminalia chebula* in the treatment of cancer.

Conclusion

In conclusion, ethanolic extract of *Terminalia Chebula* fruit significantly inhibited tumor in EAC induced cancer in swiss albino mice. This activity involves restoration of hematopoetic parameters, reduction in tumor volume and increased lifespan of the animals. These results suggest that *Terminalia Chebula* might be a good choice for the treatment of cancer. Drug seekers can use ETC as a potential agent of anticancer chemotherapy.

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