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Cytotoxic and antitumor effects of *Tribulus terrestris* L fruit methanolic extract

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ABSTRACT
Tribulus terrestris L. (Family: Zygophyllaceae) has been used extensively for various human ailments by different traditional systems of medicine including Ayurveda. Recently, the anti-proliferative effect of this plant against breast cancer cells was reported. In the present study, the cytotoxic and antitumour effects of the methanolic extract of *T. terrestris* fruits were analysed. On in vitro cytotoxicity analysis, the extract showed 50% inhibitory concentration (IC_{50}) at 380 and 420 µg/ml, for Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells, respectively. The ascites tumor induced by EAC cells was found to be decreased considerably by the oral administration of the extract and the life span of the tumour bearing mice was enhanced to 31 and 45% by 100 and 250 mg/kg b. wt. extract, respectively.

Keywords: *Tribulus terrestris*; cytotoxicity; ascites tumor.

1. Introduction
Tribulus terrestris L., belonging to the family Zygophyllaceae is a well-known shrubby plant in various traditional systems of medicine including Ayurveda, Siddha and Unani. The plant is commonly known as "Caltrops" and found all over India up to 11,000 ft. height and widely grown in Kashmir, Rajasthan and all warm regions of both hemispheres [7]. According to Traditional Chinese Medicine (TCM), the plant is considered as aphrodisiac and reported to stimulate sexual behavior of men, strengthen the heart muscle and reduce the level of cholesterin [1]. The fruit is commonly used to treat vitiligo, eye and abdominal diseases [4]. *T. terrestris* is commonly used in folklore medicine as tonic, analgesic, astringent, stomachic, anti-hypertensive, diuretic and urinary anti-infective. Sapogenins like diosgenin, gitogenin, chlorogenin was reported in the plant and the flavonoids contained in the fruit are said to be used for the treatment of gout [7].
Recently, various studies were conducted to evaluate the pharmacological properties of *T. terrestris*. The anti-arthritic activity of *T. terrestris* was analysed using Freund's complete adjuvant (FCA) induced arthritis in rats [9]. The plant extract inhibited the carrageenan-induced inflammation in rats [2] by suppressing the expression of various inflammatory cytokines [11]. The anti-cariogenic activity of this plant against *Streptococcus mutans* was reported [10]. The protective effect of tribulosin isolated from *T. terrestris* against cardiac ischemia/reperfusion injury and its underlying mechanism was also studied [17]. There is evidence for anti-proliferative effect of *T. terrestris* saponins on mouse carcinoma [5] and breast cancer [14]. The saponin fraction from *T. terrestris* was found less toxic to normal human fibroblasts [13]. The anti-carcinogenic effect of *T. terrestris* on DMBA induced papilloma in mice [8] was also reported. Considering the medicinal activity, traditional information and various experimental evidences, the present study evaluated the chemotherapeutic potential of *T. terrestris* by analyzing the cytotoxic and antitumor properties.

2. Materials and Methods
2.1 Plant materials
The fruits of *Tribulus terrestris* were collected from the Amala Ayurvedic Research Centre, Amala, Kerala and identified by Dr. P. Sujanapal, Taxonomist, Kerala Forest Research Institute, Peechi, Kerala, India.

2.2 Cell lines
Dalton's Lymphoma ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells, obtained

from Cancer Institute, Adayar, Chennai, India were maintained in the peritoneal cavity of mice and used for the study.

2.3 Animals
Swiss albino (20-30 g size) mice, purchased from the Small Animal Breeding Station, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India were maintained under standardized environmental conditions (22-28 ºC, 60-79% relative humidity, 12 h dark/light cycle) and fed with standard rat feed (Sai Durga Feeds, Bangalore, India) and water *ad libitum*. All the experiments conducted during the present study had prior permission from the Institutional Animal Ethics Committee (IAEC) and strictly followed the guidelines of Animal Ethics Committee, Government of India.

2.4 Preparation of fruit extract
The fruits of *T. terrestris* were dried in a hot air oven, powdered using a mixer grinder and extracted with methanol. The extract was filtered, concentrated, evaporated to dryness and the dried extract (TTE) was used for further studies.

2.5. *In vitro* cytotoxicity assay
Cytotoxicity of TTE was assessed using DLA and EAC cells. Briefly, 1×10^6 cells were suspended in Phosphate Buffered Saline (PBS), (0.2 M, pH 7.4) containing various concentrations of extracts in a final volume of 1 ml. The cells were incubated at 37 ˚C for 3 hr and the viability of the cells was determined by the trypan blue exclusion method [15]. Percentage of cytotoxicity was calculated by comparing with untreated control.

2.6. *In vivo* anti-tumour analysis
Approximately, 1 x 10^6 EAC cells were injected into the intra-peritoneal cavity of mice for developing ascites tumor. The animals were divided in to 5 groups according to the treatment schedule. The group 1 served as untreated control; Group 2 received cyclophosphamide (25 mg/kg body weight) as standard drug. Group 3 treated as vehicle control and received 200 µl of propylene glycol. Group 4 and 5 received two doses of TTE, 100 and 250 mg/kg b wt., respectively. The drug treatment was started the next day after the injection of cells for 10 consecutive days. The animals were observed for the development of ascites tumor and death was recorded for 30 days. The life span of the animals was calculated using the formula, percentage increase in life span (ILS) = (T-C)/C×100, where T and C are mean survival of treated and control mice.

2.7. Statistical analysis
The values are presented as mean ± SD of six animals.

3. Results
3.1 Cytotoxic analysis
TTE exhibited cytotoxic activity in a dose dependent manner. The concentration required for 50% death (IC_{50}) was found to be 380 ± 2.12 and 420 ± 5.43 μg/ml for DLA and EAC cells, respectively (Fig I).

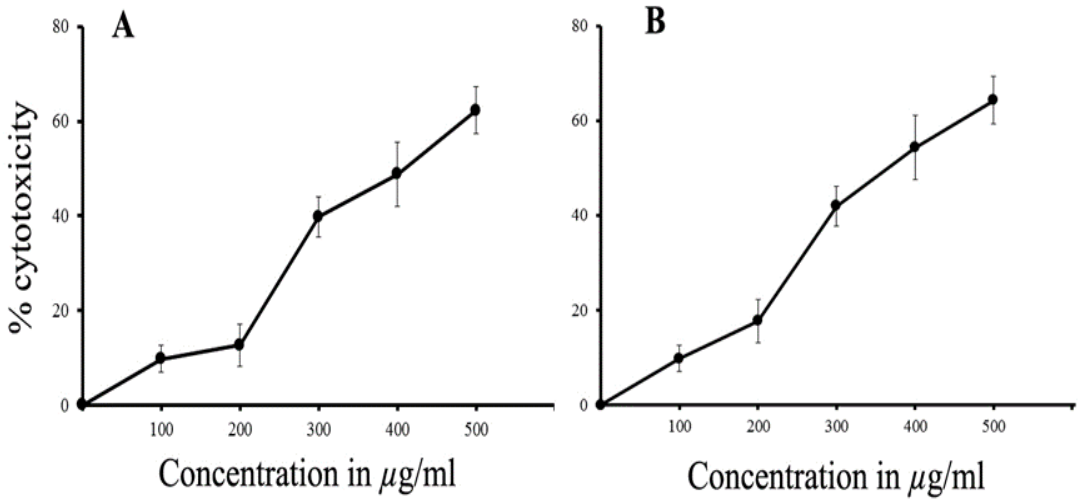

Fig 1. Cytotoxic activity of *Tribulus terrestris* fruit methanolic extract of on DLA (A) and EAC (B)

3.2 Anti-tumor analysis
The animals in the untreated control group showed remarkable ascites tumor burden and the very short survival period from the day of tumour inoculation. The vehicle control (propylene glycol) group showed no effect on hindering the proliferation of tumour cells. While, the plant extract treated groups, the animals were found to be surviving longer than that of the control group and cyclophosphamide treated animals showed higher longevity. The mean survival days for the control group was 20 ± 1.2, whereas in the treated group, it was elevated to 26.2 ± 1.4 and 29 ± 1.2 for 100 and 250 mg/kg b wt., respectively (Fig 2).

The percentage increase in life span (ILS) of the drug treated animals was calculated as 31 and 45% for 100 and 250 mg/kg, respectively. Cyclophosphamide group exhibited most significant result of 73% (Tab 1).

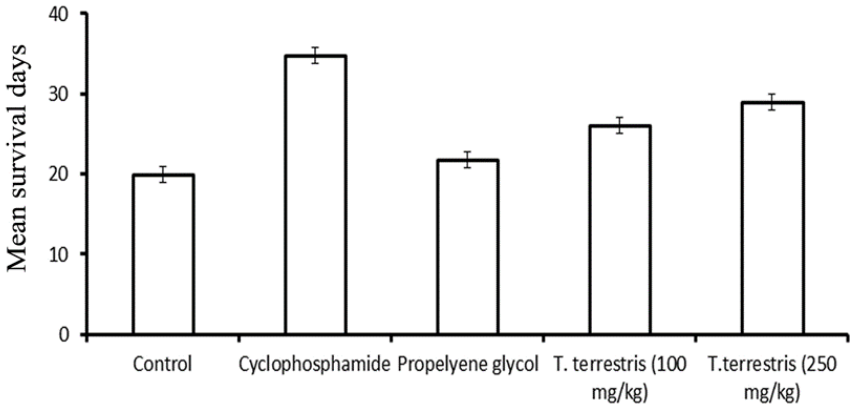

Fig 2. Effect of *Tribulus terrestris* fruit methanolic extract of on ascites tumor bearing mice

Table 1: Effect of *Tribulus terrestris* fruit methanolic extract on average life span of ascites tumor bearing mice.

Group	% of increase in Life span
Control	_
Cyclophosphamide	73
Tribulus terrestris extract (100 mg/kg)	31
Tribulus terrestris extract (250 mg/kg)	45

4. Discussion

A large number of therapeutics with low side effects is of natural origin [12]. In this respect, plants are extensively exploited as a potential source for the active components with high antitumor activity. Earlier, the cytotoxic activities of five furostanol saponins from the fruit of *T. terrestris* were studied [16]. A study conducted on aqueous extract of *T. terrestris* showed that it induces cell growth arrest and apoptosis by down-regulating NF-κB signaling in liver cancer cells [6]. In the present study, the fruit extract of *T. terrestris* showed cytotoxicity on DLA and EAC cells. In mouse ascites tumor model, the extract exhibited significant anti-tumor activity.

Even though, the cytotoxic [2] and anti-carcinogenic effect [8] of *T. terrestris* was reported, there is no reports on its antitumor activity. The results of the present study signify the antitumour potential of *T. terrestris*. This will provide the basis for the investigation for identifying novel bioactive compounds from this plant with anti-cancer potential and their mechanism of action at the molecular level. Considering the anti-tumor, anti-inflammatory and diuretic property, *T. terrestris* is suggested as a promising candidate for treatment of kidney and urinary cancers.

5. Acknowledgement

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