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In vitro cytotoxicity of *Calotropis procera* and *Trigonella foenum graecum* against human cancer cell lines

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ABSTRACT

Alcoholic whole plant extracts of Calotropis procera and Trigonella foenum graecum L. showed in vitro cytotoxicity against different human cancer cell lines such as lung, neuroblastima, liver and colon. Sulforhodamine B dye (SRB) assay was done for in vitro cytotoxicity test assay against five human cancer cell lines namely of lung (A-549), liver (Hep-2) colon (502713, HT-29) and neuroblastima (IMR-32). Cannabis sativa and Trigonella foenum graecum L. showed more than 70% growth of in some cell lines 79% and 83% in Cannabis sativa and Trigonella foenum graecum L. respectively, against lung (A-549) cell line. In case of liver (Hep-2) cell lines, Cannabis sativa showed cytotoxic activity but Trigonella foenum graecum L extracts showed no activity. Colon 502713 cell lines were inhibited by the extracts of Trigonella foenum graecum L only. HT-29 liver human cancer line and IMR-32 neuroblastima cell lines showed cytotoxic inhibition by both plant extracts.

Keywords: Human cancer cell lines, *In vitro* cytotoxicity test, Sulforhodamine B dye**,** *Calotropis procera, Trigonella foenum graecum*

INTRODUCTION

Fenugreek (*Trigonella foenum graecum* L.; family Leguminoceae) is one of the traditionally used medicinal plants. Its seeds and leaves are used not only as food but also as an ingredient in many medical formulations in traditional medicine. Seeds of fenugreek are used as a condiment and as a supplement to wheat and maize flour for bread making and as a constituent of the daily diet of general population in Indian subcontinent. Some of the therapeutic uses of *T. foenum graecum* include its use as hypoglycemic, antiulcerogenic, hypocholesterolemic and

antihypertensive agent [1]. In Ayurvedic and Unani systems of medicine, fenugreek is used for the treatment of epilepsy, paralysis, gout, dropsy, chronic cough and piles [2]. *Calotropis procera* (Gentianales: Asclepiadaceae) is an Asian shrub. Different parts of this shrub are used in medicines. Its anthelmintic [3], analgesic, antipyretic, antispasmodic, anti-inflammatory [4] and antimalaric activity against *Plasmodium falciparum* [6] are used in the treatment of many diseases. The presence of high amount of Flavonoids make the plant potentiality similar to other natural sources of vitamin C [5, 6, 7]. In the present investigation, the authors have set forth the objective of evaluating the in vitro cytotoxic activity of plant extracts against different cancer cell lines.

MATERIALS AND METHODS

Plant material and cell lines

Plant materials were collected from February to March 2008. The places of collection were from Chitrakoot region, Uttar Pradesh. The whole plants methanolic extract was used for *in vitro* cytotoxicity against different human cancer cell lines. Plant materials were dried at 37^0C, powdered and extracted in methanol. Extract was fine-filtered and freeze dried. For the preparation of the extracts, dried ground plant material was percolated with 95% methanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter sterilized (0.2µm) before testing on cell lines.

Hep-2 (liver) and HT-15 (colon) human cancer cell lines were grown in RPMI-1640 with 2 mM L-glutamine medium (pH 7.2). Penicillin was dissolved in PBS and sterilized by filtering through 0.2µ filter in laminar air flow hood. The media was stored in refrigerator (2-8^{o}C). Complete growth medium contained 10% FCS. The medium for cryopreservation contained 20% FCS and 10% DMSO in growth medium. The cell lines were maintained at 37^0C in a 5% CO_2 atmosphere with 95% humidity.

In vitro Assay for cytotoxic activity and preparation of cell suspension for assay

The anticancer activity was determined by the cytotoxic potential of the test material using human cancer cell lines which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) that binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells. The liver (hep-2) and colon (HT-15) human cancer cell lines were grown in multiple tri conical flasks (TCFs) at 37^0C in an atmosphere of 5% CO_2 and 90% relative humidity in complete growth medium to obtain enough number of cells. Cells were harvested and separated to single cell suspension by gentle pipetting action and the viable cells were counted in a hemocytometer using trypan blue. Viable cell density was adjusted to 5,000- 40,000 cells/100µl depending upon the cell line [8]. The plates containing cell suspension and complete growth medium were incubated at 37^0C for 24 hours in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. After 24 hours, the test material, vehicle control (DMSO) and positive controls were added.

Sulforhodamine B (SRB) assay

The antiproliferative SRB assay was performed to screen the growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye [9]. The microtiter plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then discarded. The plates were

washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried and SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1% acetic acid. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

RESULTS & DISCUSSION

The test sample showing growth inhibition more than 70% at 100 µg/ml were considered to be active. Against lung (A-549) cell line, *Cannabis sativa* and *Trigonella foenum graecum L* showed more than 70% growth (79% in case of *Cannabis sativa* and 83% in case of *Trigonella foenum graecum* L). Liver (Hep-2) cell lines were found to be inhibited by the extracts of *Cannabis sativa* but *Trigonella foenum graecum* L showed no cytotoxic activity. Similarly Colon cell lines were negatively affected by plant extract of *Trigonella foenum graecum L* only. In case of HT-29 liver human cancer line and IMR-32 neuroblastima cell line, both plant extract showed cytotoxic activities (Figure 1). We therefore, can conclude that plant extracts showed selective *in vitro* cytotoxity against different cancer cell lines and both the plants were found to be highly effective against various cancerous cells. The results were in accordance with the previous research done in both of the plants by other authors. Fenugreek has primarily been described as an antihyperglycemic herb in humans as well as in laboratory animals [10, 11]. Its cholesterol-reducing effect is also well established [12]. In the present study, its methanolic extract was proved to have effective cytotoxicity. Maximum growth inhibition was reported in *Trigonella foenum graecum L* against 502713 and HT-29 haman cancer cell lines. The main chemical constituents of *T. foenum graecum* are reported to be fibers, flavonoids, polysaccharides, saponins, polysaccharides and some identified alkaloids such as trigonelline and choline [13].

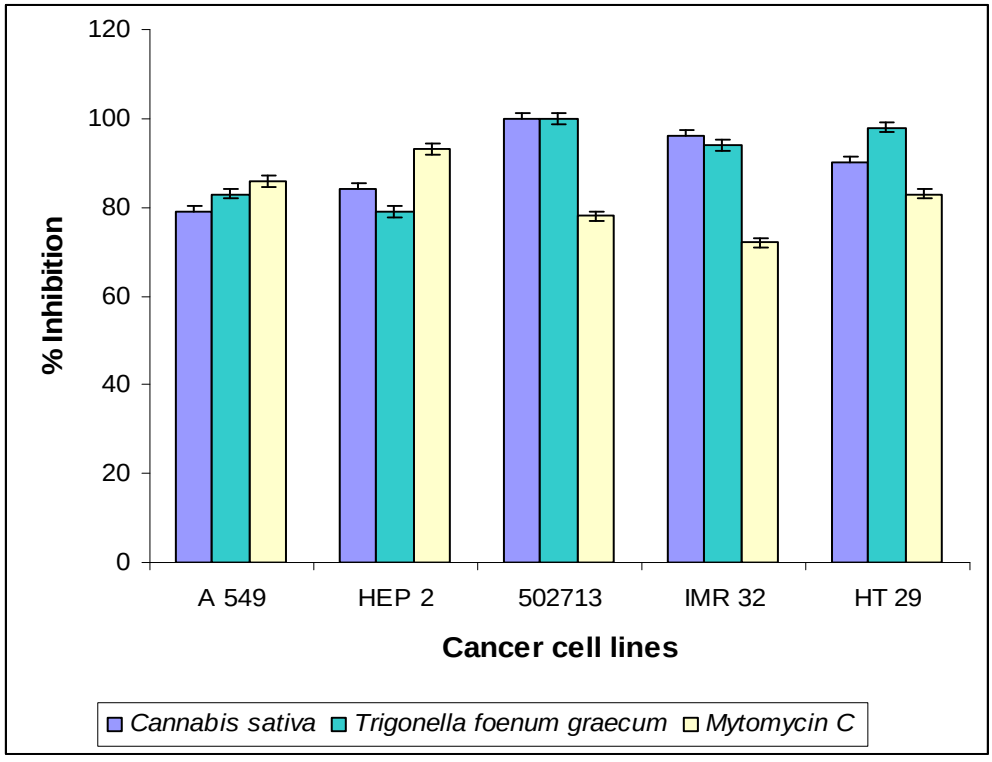

Figure 1: *In vitro* cytotoxicity tests of plant methanolic extracts against different cancer cell lines. The values represent Mean ± SE

Saponins, in particular, are described as immunostimulating agents [14]. It is suggested that some of the constituents might be having mitogenic effects, which in turn lead to stimulatory effects on the immunocompetent cells. Fenugreek seeds are rich source of dietary fibres. These fibres may not only be helpful in reducing blood sugar but their stimulatory effects on macrophages can also be not ruled out [15]. High quantity of mucilage (about 28%) reported in fenugreek also may partly be responsible for an inducing effect on macrophages. This seems to facilitate *in vitro* cytotoxity effect.

Similarly *C. procera* has been received special attention of researchers because of its high medicinal value [16, 17, 18]. Van Quaquebeke isolated a natural cardiotonic steroid from the methanolic extract of *C. procera* root barks and developed a new hemisynthetic cardenolide derivative named UNBS1450 that displayed *in vitro* antiproliferative action comparable to taxol [19]. It is established that cardiotonic steroid glycosides (bufalin and digoxin, for instance) are capable to kill cancer cells through the activation of apoptotic pathways [20, 21, 22]. Findings of the present study established that *T. foenum graecum* and *Calotropis procera* has appreciable *in vitro* cytotoxity activity. It is not possible at this juncture to single out the most effective *in vitro* cytotoxicity constituent of *T. foenum graecum* and *Calotropis procera.* However, based on the published studies, saponins, fibers, flavonoids alkaloids seem to be most likely candidates that might be responsible for eliciting the *in vitro* cytotoxicity effects of plant extracts.

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