Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Anticancer activity of *Tephrosia purpurea* and *Ficus religiosa* using MCF 7 cell lines

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ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form 11 May 2011
Accepted 15 May 2011
Available online 20 July 2011

Keywords:
Tephrosia purpurea
Ficus religiosa
MCF 7 cell line
trypan blue

ABSTRACT

Objective: To investigate anticancer activity of different fractions of *Tephrosia purpurea* [TP] (Sharapunkha, Fabaceae) and *Ficus religiosa* [FR] (Peepal, Moraceae). **Methods:** The fractions of TP and FR were prepared and tested for *in vitro* anticancer activity using human MCF 7 cell line by trypan blue exclusion method. **Results:** The result showed that among all these fractions of TPI, TPIII, FRI and FRIII showed better anticancer activity compared to other fractions. The IC_{50} value for TPI (152.4 μM), TPIII (158.71 μM), FRI (160.3 μM) and for FRIII (222.7 μM) was observed. **Conclusions:** The present study shows anticancer potential of TP and FR fractions in MCF 7 cell line.

1. Introduction

Several epidemiological studies have shown that a diet rich in vegetables and fruits might protect against cancer by mechanisms that have not been well defined yet. In recent years, naturally occurring plant products have been getting increased attention for the intervention of malignant invasive progression in the late stage of neoplastic diseases[1, 2]. On the basis of this idea, certain foods including many vegetables, fruits, and grains, as well as phytochemicals of diversified pharmacological efficacies have been shown to offer a significant protection against various cancers[3-5]. Furthermore, there is an increased focus on providing scientific basis to use these agents in the prevention strategy for people with a high risk for cancers.

Tephrosia purpurea (TP) L. (Fabaceae), commonly known as "Sharapunkha" in Sanskrit, is a copiously branched, sub-erect, herbaceous perennial plant, which occurs through out the Indian[6]. Whole plant has been used to cure tumors, ulcers, leprosy, allergic and inflammatory conditions such as rheumatism, asthma and bronchitis[7]. The aqueous extract of TP seeds has shown significant *in vivo* hypoglycemic activity in diabetic rabbits[8]. The flavanoids isolated from the plant has been reported to have antimicrobial activity[9]. It has also been reported to acquire hepatoprotective, mast cell stabilizing and erythrocyte membrane integrity enhancing effect in various animal models[10-11]. Phytochemical investigations on TP have revealed the presence of various phytoactive constituents such as glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols[12].

Ficus religiosa (FR) L. (Moraceae) commonly known as 'Peepal' is a variety of fig and sacred tree native to India. It is reported to have numerous therapeutic uses in folkmedicine viz.: leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhea, haematuria, ear-ache and toothache, migraine, eye troubles, gastric problems and scabies; leaf decoction has been used as an analgesic for toothache; fruits for the treatment of asthma, other respiratory disorders and scabies; stem bark is used in gonorrhea, bleeding, paralysis, diabetes, diarrhea, bone fracture, antiseptic, astringent and antidote[13]. In Ayurveda it is claimed that FR possesses anticonvulsant activity[14], acetyl cholinesterase inhibitory activity[15] and antianxiety activity[16]. Fruits of this plant contain numerous

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amino acids whereas figs of this plant has been reported to contain highest amount of serotonin[5-HT] as compare to figs of other *Ficus* species[17]. Phytochemical investigations on FR have revealed the presence of various phytoactive constituents such as glycosides, sterols, tannins, amino acids, campesterol, isofucosterol, stigmasterol and lupeol. Leaf extract of FR had been shown to contain high amounts of tannins, phenols, triterpenoids, glucosides and sterols[18-20].

2. Material and methods

2.1. Plant material

The leaves of TP and FR were collected from the local region in July, 2008. The plant material was identified and authenticated by Prof. P. G. Diwakar, Botanical survey of India, Pune (Voucher No. BSI/WC/Tech/08/340).

2.2. Preparation of TP fractions

The extraction was carried out using pet ether initially, then it was allowed to evaporate slowly in shallow dish and resinous mass was discarded. The remaining material was further fractionated into acetone soluble and acetone insoluble portions. These two portions were dissolved in benzene and subjected to column chromatographic separation as detailed below.

(1) Chromatographic separation of acetone soluble and insoluble part

For column chromatography Silica gel c was first activated at 150 ℃ for 3 h in an oven. After cooling, slurry was prepared in benzene, it was poured in glass column and set aside for 2 h. The residue of pet ether extract was dissolved in minimum volume of benzene and it was mixed thoroughly with silica gel. It was air dried and charged into the column. The elution was carried out first with benzene (TPI) and successively with ethyl acetate (TPII). The solvents were changed when portion of eluent showed absence of residue. The separation of acetone insoluble part was carried out as described above with benzene (TPIII) followed by ethyl acetate.

(2) Preparation of fractions of alcoholic extract

The dried alcoholic extract was separated into water soluble and water insoluble portion (TPIV) using minimum quantity of distilled water. Only about 10% extract was soluble in water, which was vigorously shaken repeatedly with small volumes of ethyl acetate (TPV) till the ethyl acetate layer becomes colorless.

2.3. Preparation of FR fractions

The extraction was carried out using pet ether followed by alcohol, then it was allowed to evaporate slowly in shallow dish and resinous mass was discarded. For column chromatography neutral alumina was first activated at 150 ℃ for 3 h in an oven. After cooling, slurry was prepared in benzene, it was poured in glass column and set aside for 2 h. The residue of pet ether extract was dissolved in minimum volume of benzene and it was mixed thoroughly with neutral alumina. It was air dried and charged into the column. The elution was carried out first with benzene (FRI) and successively eluted with ethyl acetate (FRII). The dried alcoholic extract was separated into water soluble and water insoluble portion Water soluble portion was shaken vigorously with methanol yielded a gelatinous precipitate (FRIII). The water insoluble part was dissolved in minimum volume of absolute alcohol and column chromatography was carried out with benzene (FRIV) and ethyl acetate (FRV).

2.4. Trypan blue exclusion test of cell viability

The dye trypan blue exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. In this test, a cell suspension is simply mixed with dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. In brief, an aliquot of MCF -7 cell suspension was centrifuged for 5 min at 100 × g and supernatant was discarded. The pellet formed thus resuspended in 1 mL of PBS which serves as cell suspension. 1 part of 0.4% trypan blue was mixed with 1 part cell suspension and supplemented with TPI, TPIII, FRI and FRIII fractions(0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μg/mL) allowed to incubate for 3 min at room temperature. A drop of trypan blue/cell mixture was applied to a hemacytometer and placed on the stage of a binocular microscope and focus on the cells. The unstained (viable) and stained (nonviable) cells were counted separately to obtain the total number of viable cells per ml of aliquot[21].

Viability can be calculated with the formula

$$\% \text{ Viability} = \frac{\text{Total number of living cells}}{\text{Total number of cells}} \times 100$$

3. Results

The Figure 1 and 2 showed that sharp inhibition in the cancer cell lines while neither decrease nor increase in the log cell count. It can be interpreted as the drug scaffold having inhibitory activity against the breast cancer cell lines but in case of normal cell line drug was ineffective. The drug neither allowed the cell growth nor killed the existing cell.

The IC_{50} value for TPI was found to be152.4 μM, whereas the IC_{50} value for TPIII was found to be 158.71 μM.

The graphs 3 and 4 showed inhibition in the cancer cell lines while it shows no decrease no increase in the log cell count. This shows the signs of cytotoxicity with the normal epithelial cell. The IC_{50} value for FRI was found to be 160.3 μM, whereas the IC_{50} value for FRIII was found to be 222.7 μM.

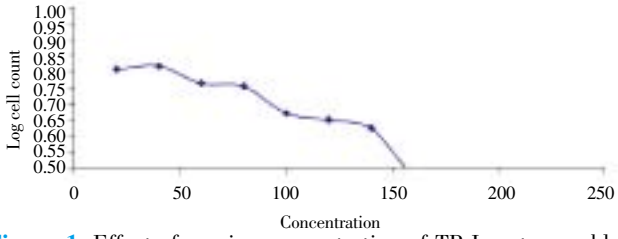

Figure 1. Effect of varying concentration of TP I on trypan blue exclusion test of cell viability in MCF 7 cell lines.

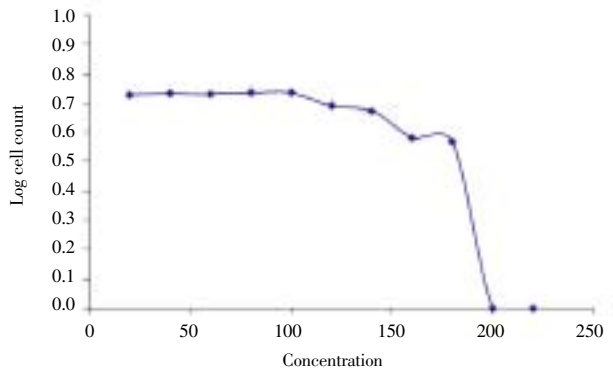

Figure 2. Effect of varying concentration of TP III on trypan blue exclusion test of cell viability in MCF 7 cell lines.

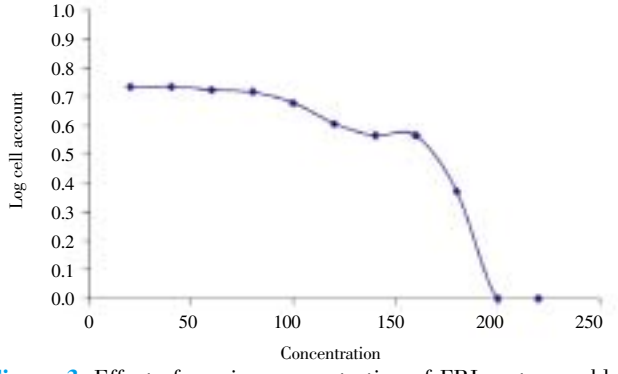

Figure 3. Effect of varying concentration of FRI on trypan blue exclusion test of cell viability in MCF 7 cell lines.

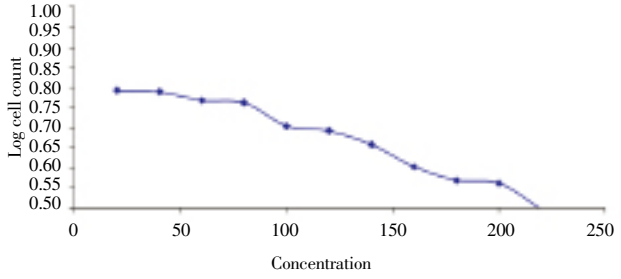

Figure 4. Effect of varying concentration of FRIII on trypan blue exclusion test of cell viability in MCF 7 cell lines.

4. Discussion

Cancer is a growing health problem around the world. Natural products have long been used to prevent and treat many diseases, including cancer and thus they are good candidates for the development of anti-cancer drugs. The large population use ayurvedic medicine worldwide, there is urgent need for additional, carefully conducted, high-quality intensive research to evaluate its efficacy and to develop this discipline to meet ever-new challenges of modern medicine in the field cancer research. Different *in vivo* and *in vitro* screening models are available for anticancer activity. In the present study, we used human breast cancer cell line such as MCF-7 cells. MCF-7 cells, which are estrogen receptor (ER)-dependent and carries the wild type tumour suppressor p53 gene[22] are tested by using trypan blue exclusion method which is based on decrease in viable tumor cell count and increased non-viable tumor cell count. The proposed study revealed that the fractions obtained from both plants were effective in attenuating the viable tumor cell count. For both fractions of TP i.e. TP I and TP III employed in this study, viable tumor cell count decreased in dose dependant manner and more predominantly at concentration of 200 μg/mL where each of these fractions inhibit all the viable tumor MCF-7 cell. Similarly, fractions obtained from Ficus religiosa named FR I and FR III also inhibits the viable cell count but not as effective as that of TP I and TP III. The concentration at which FR I and FR III inhibit the viable tumor cell count was beyond 200 μg/mL which reveals that TP fractions are more potent in inhibiting the MCF-7 cells.

Previous studies in our laboratory showed that the extracts of TP are rich in flavonoid content and the cytotoxic might be due to the presence of flavonoids in the fractions utilized in this study as flavonoids have been shown to possess antimutagenic and antimalignant effect[23-24].

Moreover, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis[25-27].

Treatment with FR fractions showed cytotoxic activity which was less potent as compare to TP. However this activity may be due to the presence of phenolic contents in the fractions. It was reported that along with antioxidant activity, this group of compounds also posses a wide variety of biological functions which are mainly related to modulation of carcinogenesis. Various in vitro and in vivo systems have been employed to determine the anticarcinogenic and anticancer potential of these natural phenolic compounds or extracts[28].

Taken together our results suggests that the fractions of extracts obtained in from TP and FR have good cytotoxic activity against human breast cancer cell line i.e. MCF-7 which may be attributed to the flavonoids and phenolic constituents of the fractions.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are thankful to Dr. Nitin Desai, Dean, Faculty of Sciences, Dr. DY Patil Deemed University, Sector15, CBD Belapur, Navi Mumbai for providing necessary facilities.

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