ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/263655573

Study of antiproliferative activity of Tinospora cordifolia extracts in normal and malignant cells

Article in Journal of Pharmacy Research · January 2010

CITATION
1

READS
86

2 authors:

Parveen Bansal
Baba Farid University of Health Sciences
176 PUBLICATIONS 841 CITATIONS
SEE PROFILE

Satya Das
All India Institute of Medical Sciences
281 PUBLICATIONS 1,216 CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Immunotoxicology of chemicals View project

ANTI TUMOUR ACTIVITY OF PHYTOCHEMICALS IN OSCC View project

All content following this page was uploaded by Satya Das on 07 July 2014.

The user has requested enhancement of the downloaded file.

Research Article
ISSN: 0974-6943

Available online through
www.jpronline.info

Study of antiproliferative activity of *Tinospora cordifolia* extracts in normal and malignant cells

Bansal P[1*], Das SN[2]
[1]*National Institute of Ayurvedic Pharmaceutical Research, Patiala, India*
[2]*Dept of Biotechnology, All India Institute of Medical Sciences, New Delhi, India*

Received on: 23-08-2009; Revised on: 01-10-2009; Accepted on: 09-01-2010

ABSTRACT
Tinospora cordifolia (Guduchi) has been used for centuries in Ayurvedic system of medicine for treating various ailments including cancer. Here, we report that treatment with *Tinosporacordifolia* extracts (TCE) *in vitro* inhibited cell proliferation and induced cell death in a dose-dependent (25-75µg/ml) and time-dependent (24-120 hours) manner in oral squamous cell carcinoma cell line along with a significant cytostatic effect. Treatment of peripheral blood mononuclear cells (PBMC) with TCE under identical conditions did not significantly affect their viability. The effectiveness of TCE in checking the growth of KB cells without affecting the growth of normal PBMC indicates that *Tinospora cordifolia* has differential effect on normal and malignant cells hence; it may have therapeutic potential in cancer.

Keywords: *Tinospora cordifolia,* anti-proliferative, KB cell line, Oral squamous cell carcinoma.

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. Cancer of the oral cavity is a common disease in Asian country and oral squamous cell carcinoma (OSCC) is clinically the most common in Indian males. It is well known that OSCC is resistant to cancer chemotherapy mediated apoptosis. Hence often chemotherapy protocol has a limited role in eradication of the disease.

Out of 121 prescription drugs in use for cancer treatment,90 are derived from plant species and 74% of these drugs were discovered by investigating a folklore claim[1-2]. Recently, a greater emphasis has been given towards the research on complementary and alternative medicine that deals with cancer management. Several studies have been conducted on herbs under a multitude of ethno botanical grounds. For example, Hartwell[3-5] has collected data on about 3000 plants, those of which possess anticancer properties and subsequently been used as potent anticancer drugs[6].

According to *Nighantu* (Ayurvedic Pharmacopoeia), *Tinospora cordifolia* commonly known as *Guduchi* is supposed to be *Amrita (*which means that rejuvenates the dead cells). The term *Amrita* refers to heavenly elixir, which was reputed to protect the celestial people from senescence and keep them eternally young. As per latest research it is known as best immunomodulator and antioxidant[7-11]. Few reports have also demonstrated a potent anticancer activity exerted by the plant extract on different types of cancer[12-14]. Some of these reports demonstrate involvement of immune system[15-18] whereas others have shown role of the extracts on radiation induced sensitivity[19-21], however none of the study demonstrated the mechanism of action and active component of *Tinospora cordifolia*.

The present study was conducted to evaluate the antiproliferative and apoptotic activity of *Tinospora cordifolia* extracts on malignant oral squamous carcinoma cells and normal PBMC was used as control in this study.

MATERIALS AND METHODS

The present study was conducted at Tumor and Molecular Immunology laboratory of Dept. of Biotechnology at All India Institute of Medical Sciences (AIIMS), New Delhi. An oral squamous cell carcinoma cell line (KB) was obtained from National Center for Cell Sciences (NCSS), Pune, India. The KB cancer cell lines were cultured as monolayers in DMEM (SIGMA-Aldrich, USA) supplemented with 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT), 100µg/ml penicillin, and 100µg/ml streptomycin (Invitrogen, Carlsbad, CA), and maintained in an incubator with a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The drug (*Tinospora cordifolia* extract) was obtained from a standard Ayurvedic Pharmacy. A stock solution of the drug was prepared in plain medium DMEM (SIGMA-Aldrich, USA) and desired concentrations (TCE1, TCE2, TCE3 with 25, 50, 75µg/ml respectively) were drawn from time to time. Methotrexate with a concentration of (40µmol/L) was used as positive control through out the study.

***Corresponding author.**
Dr.Parveen Bansal
Assistant Director (Biochemistry)
Clinical Biochemistry Lab.,
National Institute of Ayurvedic Pharmaceutical Research,
Moti Bagh Road Patiala (Pb.) India.
Tel.: + 91-175-2228361, 91-175-2212393
Mob.: 09814135703, 09914095909
E-mail: bansal66@yahoo.com

Cell Proliferation by Alamar blue Assay

The effect of TCE on the proliferative capacity of the cells was determined using Alamar Blue assay. The assay is based on oxidation-reduction potential of Alamar Blue. The internal environment of the proliferating cells is reduced that changes the colour of the compound from indigo blue to fluorescent pink. The change in colour can be measured at 570nm and 600nm, respectively. The percent reduction of Alamar Blue corresponds to percent proliferation of the cells, which is quantified by the formula given below.

The calculations were made as per the formula given below.

$$\text{\% Reduction in treated cells} = \frac{(117216x\ a - 80586x\ b)}{(155677xd - 14652xc)} \times 100$$

$$\text{\% Reduction in control cells} = \frac{(117216x\ c - 80586x\ d)}{(155677x\ f - 14652xc)} \times 100$$

Test well

a= $A_{570\ initial} - A_{570\ final}$ b= $B_{600\ initial} - B_{600\ final}$

Control well

c= $C_{570\ initial} - C_{570\ final}$ d= $D_{600\ initial} - D_{600\ final}$

Negative control well

e= $E_{570\ initial} - E_{570\ final}$ f= $F_{600\ initial} - F_{600\ final}$

Briefly, 1 x 10^4 cells per well were plated in 96-well culture plates. After overnight incubation, the cells were treated with varying concentrations of TCE (25, 50, and 75 µg/ml) for 72 and 120 hours with two replicates. Absorbance was recorded with UV Spectrophotometer ECIL-MS5608 at 570nm and at 600nm initially and after the addition of Alamar Blue till the blue colour starts changing to pink. The effect of TCE on cell proliferation was assessed as percent proliferation in test group compared with vehicle-treated control cells, which were arbitrarily assigned 100% proliferation. In this experiment methotrexate was used as positive control.

Cell viability by Trypan Blue exclusion test

The cytotoxic effects of TCE were determined using the Trypan blue dye exclusion assay. Briefly, 1 x 10^5 cells were seeded into each well of 24-well culture plates under standard culture conditions and kept overnight in an incubator at 37°C. The next day, the cells were treated with TCE (25, 50, and 75µg/ml final concentration) for 24, 48, 72, 120 and 144 hours. At the stipulated time point, the cells were harvested after brief trypsinization and the cells that had taken the dye were counted using a microscope with a hemocytometer. The cytotoxic effects of TCE are expressed as the mean percentage (±SE) of dead cells in each treatment group from two independent experiments. Normal PBMC was used as control cells in this experiment along with methotrexate as positive control.

RESULTS

1. TCE reduces viability of KB cells

The effect of TCE was determined on the viability of the KB cell lines using the Trypan blue dye exclusion assay. Treatment of KB cells with TCE at concentrations of 25, 50 and 75µg/ml for 24, 48, and 72 and 120 hours resulted in significant cell death at 120 hr interval with a dose of 50µg/ml (Chi square trend=45.03 at $P<0.001$) (Fig 1). Further, it was tested whether TCE has any toxic effect on normal PBMC but no significant cytotoxic effect or cell death by TCE was found even after 120 hrs duration treatments with a concentration of TCE 25, 50 and 75µg/ml (at P value>0.05). Moreover, the TCE-induced death of PBMC at 50µg/ml dose and 120 hrs duration time point was significantly less ($P<0.001$) than the effects of the same dose of TCE on KB cells at the same time point (Fig 2). Thus, TCE seems to be capable of exerting a cytotoxic effect on KB cells without incurring cytotoxicity to normal PBMC under the present experimental conditions.

Figure.1 Effect of different concentrations of drug on viability (%) of KB cells

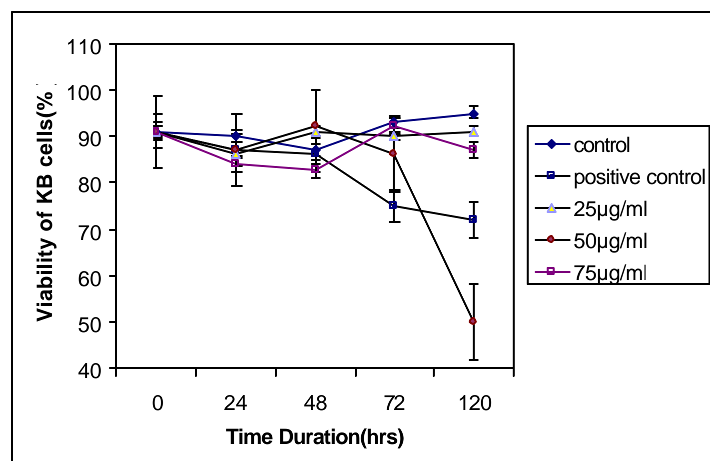


Figure.2 Effect of different concentrations of drug on viability (%) of PBMC

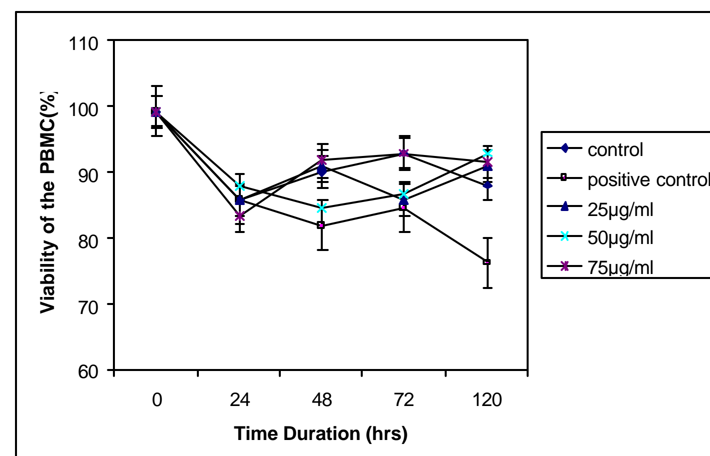


Figure.3 Effect of different concentrations of drug on total cell count at different time intervals

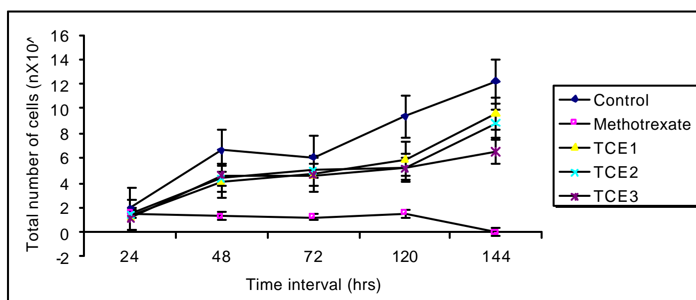
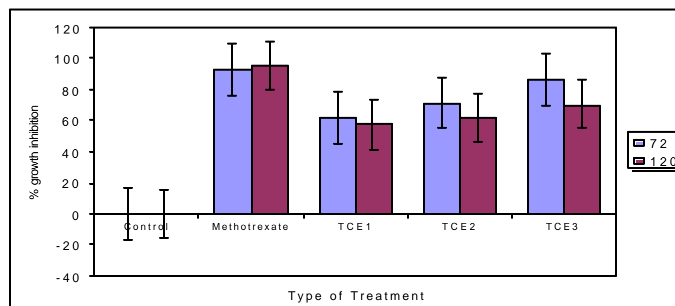


Figure.4 Effect of different concentrations of drug at different time interval on growth inhibition (%)



2. TCE induces cytostatic effects in KB Cells

Total cell count assay was performed to know the cytostatic effect of the drug. The results indicate that there was significant percent reduction in number of cells in treated KB cells as compared to the untreated control cells at p value <0.001 at all concentrations (Fig 3). Reduction in number of cells was observed at all the three selected doses demonstrating the maximum effect at 120 hrs stage at all doses TCE1 (37.24%), TCE2 (44.69%) and TCE3 (44.69%) respectively, whereas the effect accentuated in TCE3 treated KB cells at 144 hrs interval where the % reduction in cells was 46.73% as compared to 84.05% in methotrexate treated KB cells. Whenever the time the chi-square trend was applied to see the reduction in various treatments, it was observed that in methotrexate significant reduction was there at 48hrs and after that it attains constant value whereas in TCE1, TCE2 and TCE3 reduction is not significant (P>0.05). This depicts the cytostatic effect of the drug.

3. TCE inhibits proliferation and viability and induces the death of KB Cells but not of normal PBMC.

Treatment of KB cells with TCE (25, 50 and 75µg/ml) resulted in a significant reduction (p< .001 at each concentration) in cell proliferation as compared to control (Fig 4). The growth inhibition was dose dependent as at lower dose (25µg/ml) only 62.17% inhibition was observed in comparison to 71.46% and 87% inhibition at higher doses of 50 and 75µg/ml respectively, after 72hrs of incubation. At 120 hrs of incubation the effect of all these concentrations was relatively lower than that of the 72 hours treatment. A positive control of methotrexate with (40µmol/L) concentration was used which also depicted a significant reduction in proliferation of 93.24% and 95.20% (P< .001) at 72 and 120 hrs respectively (Fig.4).

DISCUSSION

The evaluation of ancient herbal medicines may indicate novel strategies for the treatment of cancer, which remains the leading cause of cancer-related deaths all over the world. In our present investigation, we show that naturally occurring *Tinospora cordifolia* extracts significantly inhibits the proliferation and reduces the viability of KB cells, which suggests that it may be an effective chemotherapeutic agent against oral cancer cells. Importantly, we found that TCE did not exhibit toxicity to PBM cells under the conditions used, except for a moderate reduction in cell viability at higher concentrations (250µg/ml) when cells were treated *in vitro* for an extended period of time (120 hrs). There was a decline in the number of total cells, similar to the results of Jagetia, Thippeswamy and Salimath[22-23] who have shown a dose-dependent decline in the clonogenicity of Ehrlich ascites tumor (EAT) cells after treatment with different concentrations of *Tinospora cordifolia*.

Control of cell cycle progression in cancer cells is considered to be a potentially effective strategy for the control of tumor growth[24-25] as the molecular analyses of human cancers have revealed that cell cycle regulators are frequently mutated in most common malignancies[26-27]. Our *in vitro* data indicated that treatment of KB cells with TCE resulted in significant G_1-phase arrest of cell cycle progression, which indicates that one of the mechanisms by which TCE may act to inhibit the proliferation of cancer cells is inhibition of cell cycle progression. The results of this study are similar to the results obtained by Thippeswamy and Salimath[23] in which TcHf (Hexane extracts of Tinospora cordifolia) decreased cell number and inhibited the proliferation of Ehrlich ascites tumor (EAT) cells by blocking cell cycle progression in the G_1-phase. G_1-phase arrest of cell cycle progression provides an opportunity for cells to either undergo repair mechanisms or follow the apoptotic pathway. Similarly berberine, a naturally occurring isoquinoline alkaloid, produced by *Tinospora cordifolia*, has been shown to exert potent anti-inflammatory and antitumor effects in *in vitro* as well as *in vivo* systems. Berberine-induced antiproliferative effects against prostate carcinoma cells was associated with G_1-phase arrest that correlated with inhibition of expression of cyclins D1, D2 and E; cyclin dependent kinases (Cdk 2, Cdk4 and Cdk6) and increased expression of Cdk inhibitory proteins p21Cip1 and p27Kip1[12].

A number of mechanisms including alteration of antioxidant activity, expression of some apoptotic proteins and immune status linked parameters have been attributed to antiproliferative effects of TCE. But from the results of present study, it seems that *Tinospora cordifolia* exerts its antiproliferative activity probably by arresting the G_1-phase of cell cycle.

CONCLUSION

Results of present investigation indicate that *Tinospora cordifolia* has the capacity to exert toxic as well as cytostatic effect on human oral squamous carcinoma (KB) cells. *Tinospora cordifolia* exerted cytotoxic effect at an optimum dose of 50µg/ml at a time interval of 120 hrs. It exerts its cytotoxic effect on KB cells without affecting normal PBMC. The evaluation of ancient herbal medicines may indicate novel strategies for the treatment of prostate cancer, which remains the leading cause of cancer-related deaths all over the world. In our present investigation, we show that a naturally occurring *Tinospora cordifolia* extracts significantly inhibit the proliferation and reduces the viability of KB cells, which suggests that TCE may be an effective chemotherapeutic candidate for human carcinomas.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge financial support by W.H.O. and laboratory facilities by Dept. of Biotechnology, AIIMS, New Delhi, India.

REFERENCES

1. Craig WJ, Phytochemicals: guardians of our health, J Am. Diet Assoc., 97, 1997, S199–204.
2. Craig WJ, Health-promoting properties of common herbs, Am. J Clin. Nutr, 70, 1999, S491–499.
3. Hartwell JL, Plants used against cancer- A survey, Lloydia, 32,1969, 78–296.
4. Hartwell JL, Plants used against cancer- A survey, Lloydia, 33,1970, 97–392.
5. Hartwell JL, Plants used against cancer-A survey, Lloydia, 34,1971, 103–425.
6. Pandey G, Anticancer herbal drugs of India with special reference to Ayurveda, Sri Satguru Publications, New Delhi, 2002, 18–121.
7. Shrivastava P, Singh SM, Singh N, Effect of thymosin alpha 1 on the antitumor activity of tumor-associated macrophage-derived dendritic cells, J Biomed. Sci., 11(5), 2004, 623-630.
8. Shrivastava P, Singh SM, Singh N, Antitumor activation of peritoneal macrophages by thymosin alpha-1, Cancer Invest., 23(4), 2005, 316-322.
9. Singh N , Singh SM , Shrivastava P, Immunomodulatory and antitumor actions of medicinal plant *Tinospora cordifolia* are mediated through activation of tumor-associated macrophages, Immunopharmacol Immunotoxicol., 26(1), 2004, 145-162.
10. Singh N, Singh SM, Shrivastava P, Effect of *Tinospora cordifolia* on the antitumor activity of tumor-associated macrophages-derived dendritic cells, Immunopharmacol Immunotoxicol., 27(1), 2005, 1-14.
11. Singh SM, Singh N, Shrivastava P, Effect of alcoholic extract of Ayurvedic herb *Tinospora cordifolia* on the proliferation and myeloid differentiation of bone marrow precursor cells in a tumor-bearing host, Fitoterapia, 77(1), 2006, 1-11.
12. Mantena SK, Sharma SD, Katiyar SK, Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3- dependent apoptosis in human prostate carcinoma cells, Mol. Cancer Ther., 5, 2006, 296-308.
13. Jagetia GC, Rao SK, Evaluation of cytotoxic effects of dichloromethane extract of Guduchi (*Tinospora cordifolia* Miers ex hook F & THOMS) on cultured HeLa cells, Evid. Based Complement. Alternat. Med., 3(2), 2006a, 267-272.
14. Jagetia GC, Rao SK, Evaluation of the Antineoplastic Activity of Guduchi (*Tinospora cordifolia*) in Ehrlich Ascites Carcinoma Bearing Mice. Biological & Pharmaceutical Bullet, 29(3), 2006b, 460.
15. SohiniYR, Bhatt RM, Activity of crude extracts formulation in experimental hepatic amoebiasis and in immunomodulation studies, J Ethnopharmacol., 54, 1996, 119-124.
16. Kapil A, Sharma S, Immunopotentiating compounds from *Tinospora cordifolia,* J. Ethnopharmacol., 58, 1997, 89-95.
17. Mathew S, Kuttan G, Immunomodulatory and antitumor activities of *Tinospora cordifolia*, Fitoterapia, 70, 1999, 35-43.
18. Balachandran P, Govindrajan R, Cancer an Ayurvedic perspective, Pharmacological Research, 51, 2005, 19-30.
19. Pahadiya S, Sharma J, Alteration of lethal effects of gamma rays in Swiss albino mice by *Tinospora cordifolia,* Phytotherapy Research, 17(5), 2003, 552-554.
20. Goel HC, Prasad J, Singh S, Sagar RK, Agrawala PK, Bala M, Sinha AK, Dogra R, Radio protective potential of an herbal extract of *Tinospora cordifolia*, J Radiat. Res. (Tokyo), 45(1), 2004, 61-68.
21. Singh L, Tyagi S, Rizvi M, Goel H, Effect of *Tinospora Cordifolia* on Gamma ray-induced perturbations in macrophages and splenocytes, J. Radiat Res (Tokyo), 48(4), 2007, 305-315.
22. Jagetia GC, Nayak V, Vidhyasagar MS, Effect of Coccinia *indica* on blood glucose, insulin and key hepatic enzymes in experimental diabetes, Pharmaceutical Biology, 40(3), 2002, 179-188.
23. Thippeswamy G, Salimath BP, Induction of caspase-3 activated DNase mediated apoptosis by hexane fraction of *Tinospora cordifolia* in EAT cells, Environmental Toxicology and Pharmacology, 23(2), 2007, 212-220.
24. Grana X, Reddy P, Cell cycle control in mammalian cells: role of cyclins, cyclin-dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CDKIs), Oncogene, 11, 1995, 211–219.
25. Pavletich NP, Mechanisms of cyclin-dependent kinase regulation: structures of cdks, their cyclin activators, and CIP and INK4 inhibitors, J. Mol. Biol., 287,1999, 821–828.
26. Kastan MB, Canman CE, Leonard CJ, P53 cell cycle control and apoptosis: implications for cancer, Cancer Metastasis Rev., 14, 1995, 3–15.
27. Molinari M, Cell cycle checkpoints and their inactivation in human cancer, Cell Prolif., 33, 2000, 261–274.

Source of support: Nil, Conflict of interest: None Declared

View publication stats