In vitro anticancer activity of ethanolic extract of *Cynodon dactylon* against HT-29 cell line

Kanimozhi D and V. Ratha Bai*

Department of Zoology, Presidency College, Chennai-600 005, Tamil Nadu, India

*Corresponding author: E-mail: kanphd5@gmail.com; Phone: +91-9884544544

Abstract

The aim of the present study is to evaluate the effect of *in vitro* anticancer activity of the ethanolic extract of *Cynodon dactylon* against HT-29 human colon cancer cell line and it was compared with normal, Vero cell line using MTT assay showed a percentage of cell viability of 97% at 0.078 mg/ml which decrease with increase in concentration of extract. Anticancer activity of ethanolic extract of *Cynodon dactylon* on HT-29 human colon cancer cell line showed potent cytotoxic activity. The inhibition percentage with regard to cytotoxicity was found to be 78.1% at 10 mg/ml, which was comparable to the control Cyclophosphamide that showed a cytotoxicity of 82%. Therefore the minimum effective concentration of ethanol extract of *Cynodon dactylon* was non-toxic to Vero cells but toxic to HT-29 cells (IC50) was recorded at a concentration of 0.625 mg/ml of the ethanolic extract of *Cynodon dactylon*.

Keywords: *Cynodon dactylon*, HT-29, MTT assay, DNA fragmentation

Received: 25th August; Revised: 16th October; Accepted: 20th November; © IJCS New Liberty Group 2013

Introduction

Plant derived agents are being used for the treatment of cancer. Several anticancer agents from plants include, taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents. *Scutellaria baicalensis* was used as a component of PCSPES, an herbal mixture that showed efficacy in laboratory trials for prostate cancer, small-cell lung cancer and acute myeloid leukemia (Cordell, 2002; Hsieh et al., 2002; Meyer and Giallatt, 2002; Ikezoe et al., 2003; Chung et al., 2004; Oh et al., 2004). Although more than 1500 anticancer drugs are in active development with over 500 of the drugs under clinical phytomedicines has increased dramatically in the last two decades (Rao et al., 2004). It has been also reported (Rosangkima and Prasad, 2004) that more than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various cancer cells of human originals, there is an urgent need to develop much effective and less toxic drugs (Chong et al., 2009).

Geinstien in plants such as parsley and soy foods inhibits protein tyrosine kinase, thereby disrupting signal transduction and inducing cell differentiation (Markovits et al., 1989; Lopez-Lazaro et al., 2007). *Cynodon dactylon* Pers. belongs to the family of Poaceae (Harlan, 1970) and is said to have many medicinal properties including anti-helmentic
(Sujon et al., 2008), antidiuretic, antiinflammatory, hepatoprotective activity (Singh et al., 2009) as well as treatment of Urinary tract infections (Cheryl, 2006), Prostatitis, and dysentery. Traditionally it is used indiabetes (Singh et al., 2007; Jarald et al., 2008) jaundice, kidney problems (Khajavi et al., 2011), urinary disease, gastrointestinal disorder (Das and Datta Choudary, 2010), Constipation and abdominal pain. The whole plant is used for diuretic, dropsy, syphilis, wound infection and piles. Cynodon dactylon is used as antihaemorrhagic in dysentery and nasal bleeding (Kunja et al., 2012). The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is used in the treatment of catarrhal ophthalmia, hysteria, epilepsy, insanity and chronic diarrhea.

The plant is folk remedy for anasarca, calculus, carbuncles, cough, hypertension, snake bites, gout and rheumatic affections. Cynodon dactylon is a valuable herbal medicine and used for first aid for minor injuries (Oudhia, 1999; Oudhia, 1999). Cynodon dactylon is bitter, sharp hot taste, good odor, laxative, brain and heart tonic, aphrodisiac, expectorant, carminative and useful against grippe in children and for pains, inflammations, and toothache (Agharkar, 1991). Virus-affected discolored leaves of Cynodon are used for the treatment of liver complaints. In homoeopathic systems of medicine, it is used to treat all types of bleeding and skin troubles (Oudhia, 1998). The ethanolic extract of aerial parts of C. dactylon showed marked protection against convulsions induced by chemo convulsive agents in mice (Dilip Kumar Pal, 2009). Ethanolic extract of defatted C. dactylon has high antidiabetic potential along with good hypolipidemic profile (Santhosh kumar singh et al., 2007). This suggests the potential for Cynodon dactylon to become an alternative to current diabetes medications. The methanolic extract of Cynodon dactylon possessed significant antitumor activity and hepatoprotective effect against Ehrlich ascitic Lymphoma (ELA) in Swiss albino mice and brought back the altered levels of the hematological parameters and liver enzymes (Saroja Marappan and Annapoorni, 2012). Aqueous and ethanol extract of C. dactylon (500 µg/ml) were investigated for their antibacterial activity against gram positive bacteria and gram negative bacteria using disc diffusion, well in agar and microdilution method. E. coli, B. subtilis, S. aureus and A. hydrophila were more susceptible in the ethanolic extract and no result was found in aqueous extract (Kaleeswaran et al., 2010). There is previous report on the anticancer activity of methanolic extract of Cynodon dactylon in COLO320 DM cells and experimentally induced colon carcinogenesis in rats (Albert Baskar and Ignachimuthu, 2010). HT-29 a cultured human colon cancer cell line is used to study at the cellular level the effect of fermented milks on colon cancer cell growth and differentiation characteristics. Undifferentiated HT-29 cells have been grown in the continuous presence of milks fermented by one of the following bacterial populations: Lactobacillus helveticus, Bifido-bacterium, Lacidophilus or a mix of Streptococcus thermo-philus and Lbulgaricus. Penicillin G was added to the cell culture medium, resulting in a complete blockade of bacterial growth without significant effect on bacterial viability (Laurent Baricutt, 1995).

Materials and Methods

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories
Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from (Sisco Research Laboratory Chemicals, Mumbai, India). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai, India.

**Media and Cell lines**

African Green Monkey Kidney (VERO) cell lines and HT29 were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 µg/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 μg/ml CO₂ at 37 °C.

**Collection of Plant material**

*Cynodon dactylon* (includes leaf, stem and root) were collected from in and around Maduravoyal region, the voucher specimen were kept in Department of Zoology, Presidency College (Chennai, India) used for this study.

**Preparation of ethanol extract**

The 25 g of dried powder of *Cynodon dactylon* was mixed with 100 ml of ethanol solvent and kept in rotary shaker at 100 rpm overnight and filtered with Whatmann no.1 filter paper and concentrated to dryness at 40°C. until further use. Different concentration of the ethanolic extracts such as 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10 mg/ml respectively were prepared in 5% Di-Methyl Sulfoxide (DMSO) to determine the cytotoxic effect. The yield of the extract was 1.97 g. The crude extract was then dissolved in 10% water in methanol.

**Experimental design**

A cytotoxicity property of ethanol extract of *Cynodon dactylon* was carried out by MTT method against HT-29 cell line and Vero normal cell.

**Cell viability assay on vero cells**

The Cytotoxicity of samples on VERO was determined by the MTT assay (Mosmann Tim, 1983). Cells (1 × 10⁵/well) were plated in 100 µl of medium/well in 96 well plates (Costar Corning, Rochester, New York, USA). After 48 hrs incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48 h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH7.4), 20 µl/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazoly)-2,5- diphenyl-tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4 h incubation, 0.04 M HCl/isopropanol was added. Viable cells were determined by the absorbance at 450 nm. Measurements were performed and the concentration required for a 50% inhibition of viability was determined graphically. The absorbance at 450 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of VERO cells was expressed as the % cell viability, using the following formula:

\[ \% \text{ cell viability} = \frac{A_{450} \text{ of treated cells}}{A_{450} \text{ of control cells}} \times 100\% . \]

**Cell viability on HT-29 cell lines**

The anticancer activity of ethanolic extract of *Cynodon dactylon* was performed on HT-29 cell lines obtained from NCCLS, Pune, India. The cell viability was measured using MTT assay as described above. Controls were maintained throughout the experiment. The assay was performed in triplicates for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract. Cells and % viability was plotted against
concentration of the plant extract. The maximum concentration of the plant extract that was non toxic to vero cells but toxic to HT-29 was recorded as the effective drug concentration.

**DNA Fragmentation technique**

The ethanolic extract which is cultured with the HT-29 cell lines was passed to DNA fragmentation technique. A distinctive feature of apoptosis at the biochemical level is DNA fragmentation (Walker et al., 1993). This method was used as a semi-quantitative method for measuring apoptosis (Wyllie et al., 1980). The culture medium was removed and centrifuged at 3000 x g for 5 min to collect detached cells. 2 ml of cells which is centrifuged to 3000 rpm suspended in 200 µL of 1X TE Buffer and 100 µL of 10% SDS, incubated at 60°C for 20 min. add 300 µL of Phenol: Chloroform: Isoamyl alcohol (25:24:1) mixed well, then centrifuge at 10,000 rpm for 10 min. To the supernatant add 500 µL of isopropanol. Add 200 µL of 70% ethanol, then centrifuge at 10,000 rpm for 10 minutes. Dry the pellet at 37°C till there are no traces of solution. Resuspend the pellet in 20 µL of 1xTE Buffer. Electrophoresis is extracted DNA on 1% agarose gel. Agarose gel electrophoresis is carriedout (Kokileva et al., 1989). For casting 1% Agarose gel add 0.8 gm of Agarose in 80 mL of diluted 1X TBE buffer. Allow the gel to solidify without disturbing the wells. Transfer the gel to 1X TBE buffer filled electrophoresis tank. Add 2 µL of gel loading dye to 20 µL of sample DNA, mix well, and then load the total 22 µL of sample to gel. Connect the power card terminals at respective positions, run the gel at 50 V till the Gel loading dye migrates more than half of the length of gel. Then switch off the unit, visualize the separated sample DNA with MW marker under UV Transilluminator.

**Results and Discussion**

The nontoxic dose of the ethanol extract of *Cynodon dactylon* on normal vero cell line showed that the percentage with regard to viability of cells was found to be 97% at a concentration of 0.078 mg/ml which decreased with increase in concentration (Table 1). The extract showed a potential cytotoxic activity against HT-29 colon cancer cell line (Table 1). Cyclophosphamide served as positive control and 82% cancer inhibition was observed (Table 2 and Fig. 2d). The concentration of ethanolic extract of *Cynodon dactylon* at 10 mg/ml showed an inhibition of 78.1% compared to that of positive control. Ethanolic extract of *Cynodon dactylon* at 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg/ml showed cytotoxic activity of 64.3%, 60.1%, 54.8%, 52.4%, 42.1%, 32%, 18.6% respectively. Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control (Fig. 2). These observations may be due to the presence of active biological compounds. Therefore the minimum effective concentration of ethanol extract of *Cynodon dactylon* that was non toxic to Vero cells, but toxic to 50% HT-29 colorectal cancer cells was recorded (IC₅₀) at a concentration of 0.625 mg/ml of the plant extract.

**DNA Fragmentation technique**

DNA fragmentation was obtained by agarose gel electrophoresis of DNA extracts from HT-29 cancer cell lines. The DNA migrated as discrete bands which by comparision to DNA markers, gave a ladder of approximately 200 base pair
Such DNA ladders are considered to be a hallmark of apoptosis (Walker et al., 1993). The ladder result from DNA fragmentation catalyzed by an endogenous endonuclease that cleaves internucleosomal DNA to form ladder like bands of oligo nucleosome fragments separated by approximately 200 bp (Wyllie et al., 1980; Kokileva, 1989).

**Fig. 2.** a. Photomicrograph of HT29 cell line. b. Cells treated with 10 mg/ml of ethanol extract of *Cynodon dactylon*. c. Cells treated with 0.625 mg/ml of ethanol extract of *Cynodon dactylon*. d. Cyclophosphamide positive control.

**Fig. 3.** DNA laddering visualized in agarose gel by ethidium bromide staining. a) Lane 1: HT-29 cell line with ethanol extract of *Cynodon dactylon*. b) Lane 2: control. c) Lane 3: kb marker.
Table 1. Cell viability assay on vero and HT-29 cell line

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Dilution</th>
<th>% cell viability of vero cells</th>
<th>% cell viability of HT-29 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.078</td>
<td>1:64</td>
<td>97</td>
<td>81.4</td>
</tr>
<tr>
<td>0.156</td>
<td>1:32</td>
<td>94</td>
<td>68.0</td>
</tr>
<tr>
<td>0.312</td>
<td>1:16</td>
<td>92</td>
<td>57.9</td>
</tr>
<tr>
<td>0.625</td>
<td>1:8</td>
<td>89</td>
<td>52.6</td>
</tr>
<tr>
<td>1.25</td>
<td>1:4</td>
<td>86</td>
<td>45.2</td>
</tr>
<tr>
<td>2.5</td>
<td>1:2</td>
<td>84</td>
<td>39.9</td>
</tr>
<tr>
<td>5</td>
<td>1:1</td>
<td>82</td>
<td>35.7</td>
</tr>
<tr>
<td>10</td>
<td>Neat</td>
<td>79</td>
<td>21.9</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-</td>
<td>-</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Conclusion

The results of this study support the efficacy of *Cynodon dactylon* as an anticancer agent for HT-29 colon cancer cell line. From the present study it has been revealed that ethanolic extract of *Cynodon dactylon* shows 50% anticancer activity in HT-29 colon cancer cell line at the concentration of 0.625 mg/ml. It acts a potential adjuvant treatment to current chemotherapeutic agents and can be used in the treatment of HT-29 colon and a further research has to be done. From this it is said that due to the presence of some anticancer components, it shows 50% activity. In future the components present on *Cynodon dactylon* may act as a drug, further *in vivo* studies should be carried out. Considerable works have been done on the medicinal plants to treat cancer, and some plant products have been marketed as anticancer drugs. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. Several reports describe that the anticancer activity of these plants is due to presence of antioxidants.

References


Dilip Kumar Pal (2009). Determination of Brain Biogenic Amines in *Cynodon dactylon* pers & *Cyperus rotundus*


