Research article Available online www.ijsrr.org ISSN: 2279-0543

International Journal of Scientific Research and Reviews

# In-Vitro Anticancer Activity of Ethanolic Extract of *Cynodon dactylon* Against HEP-2, HELA and MCF-7 Cell Lines.

Kanimozhi D*

Department of Zoology, Presidency College, Chennai-600 005, Tamilnadu, India.

## 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 HEP-2 laryngeal, HELA cervical and MCF-7 breast cancer cell lines and it was compared with normal, Vero cell line using MTT assay showed a percentage of cell viability of 97 % at 0.078mg/ml which decrease with increase in concentration of extract. Anticancer activity of ethanolic extract of *Cynodon dactylon* on HEP-2, HELA and MCF-7 cancer cell lines showed potent cytotoxic activity. The inhibition percentage with regard to cytotoxicity was found to be 93.5%, 88.5% and 79.2% at 10mg/ml, which was comparable to the control Cyclophosphamide that showed a cytotoxicity of 96%, 92% and 83% Therefore the minimum effective concentration of ethanol extract of *Cynodon dactylon* was non-toxic to Vero cells but toxic to HEP-2, HELA and MCF-7 cells(Ic50) was recorded at a concentration of 0.156mg/ml 0.625mg/ml of the ethanolic extract of *Cynodon dactylon*. Among these three cell lines *Cynodon dactylon* shows more activity in HEP-2 laryngeal cell line.

**KEYWORDS:** *Cynodon dactylon,* HEP-2, MCF-7 cell lines, MTT assay, DNA Fragmentation

## *Corresponding Author:

Kanimozhi.D
Department of Zoology, Presidency College,
Chennai-600 005, Tamilnadu, India.
Email: kanphd5@gmail.com,
Telephone: 09884544544

## 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 epipodo phyllotoxin 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[1-6]. 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[7] . It has been also reported[8] 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 . Invitro studies[9]. Geinstien in plants such as parsley and soy foods inhibits protein tyrosine kinase, thereby disrupting signal transduction and inducing cell differentiation[10,11].

*Cynodon dactylon* .Pers. belongs to the family of Poaceae[12] and is said to have many medicinal properties including Antihelmentic[13], Antidiuretic, Antiinflammatory, Hepatoprotective activity[14] as well as treatment of Urinary tract infections[15], Prostatitis, and Dysentery. Traditionally it is used in diabetes[16,17] jaundice , kidney problems[18], urinary disease, gastrointestinal disorder[19], 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[20]. The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is used in the treatment of catarrhal opthalmia, 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[21,22].*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[23].

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[24]. The Ethanolic extract of aerial parts of *C.dactylon* showed marked protection against convulsions induced by chemo convulsive agents in mice[25]. Ethanolic extract of defatted *C. dactylon* has high antidiabetic

potential along with good hypolipidemic profile[26]. 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[27]. Aqueous and ethanolic extract of *C. dactylon* (500µg/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[28]. *Invitro* cytotoxic of the root extract of *Rubia cordifolia* exhibited significant cytotoxic activity against Hep-2 cell line[29] . The fruits of *Solanum nigrum* methanolic extract were tested for its inhibitory effect on HeLa Cell Line. The cytotoxicity of *Solanum nigrum* on HeLa cell was evaluated by the SRB assay and MTT assay. *Solanum nigrum* methanolic extract has significant cytotoxicity effect on HeLa Cell Line in concentration range between10mg/ml to 0.0196mg/ml by using SRB assay and study also showed that inhibitory action on HeLa cell line in concentration range between 10 mg/ml to 0.0196mg/ml by using MTT assay[30]. The antioxidant and anticancer activities were assessed for two Bangladeshi ginger varieties (Fulbaria and Syedpuri) at young age grown under ambient (400 mol/mol) and elevated (800 mol/mol) $CO_2$ concentrations against two human breast cancer cell lines (MCF-7 and MDA-MB-231)[31].

## 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). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai

### *Media and Cell lines:*

African Green Monkey Kidney Normal Vero cell, Hep-2, Hela and Mcf-7 cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100µg/ml), and streptomycin (100 g/ml) in a humidified atmosphere of 50 g/ml $CO_2$ at 37°C.

## *Collection of Plant material:*

*Cynodon dactylon* were collected from in and around Maduravoyal region, The voucher specimen were kept in the Department of Zoology, Chennai, Tamilnadu , India and used for this study.

## *Preparation of ethanol extract:*

25g of dried powder of *Cynodon dactylon*(include leaf, stem and root) ,was mixed with 100ml of ethanol solvent and kept in rotary shaker at 100 rpm overnight and filtered with whatman no.1 filter paper and concentrated to dryness at $40^0$c. until further use. Different concentration of the ethanolic extracts(0.078mg/ml, 0.156mg/ml, 0.312mg/ml, 0.625, 1.25mg/ml, 2.5mg/ml, 5mg/ml, 10mg/ml) were prepared in 5% Dimethyl Sulfoxide (DMSO) for determining cytotoxicity. The yield of the extract was 1.97g.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, HEP-2 Laryngeal, HELA Cervical and MCF-7 Breast cancer cell lines and Vero normal cell.

## *Cell viability assay on vero cells:*

The Cytotoxicity of samples on VERO was determined by the MTT assay[32]. Cells ($1 \times 10^5$/well) were plated in 100 l of medium/well in 96-well plates (Costar Corning, Rochester,NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/isopropanol was added. Viable cells were determined by the absorbance at 450nm. 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:

% cell viability = A450 of treated cells / A450 of control cells × 100%.

### *Cell viability on Hep-2, HeLa and Mcf-7 Cancer cell lines:*

The anticancer activity of ethanolic extract of *Cynodon dactylon* was performed on Hep-2 laryngeal, HeLa Cervical and Mcf-7 Breast cancer 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 Hep-2, HeLa and Mcf-7 cell lines was recorded as the effective drug concentration.

### *DNA fragmentation technique:*

The ethanolic extract of *Cynodon dactylon* which is treated with Hep-2, HeLa and Mcf-7 cell lines was passed to DNA fragmentation technique. A distinctive feature of apoptosis at the biochemical level is DNA fragmentation[33]. This method was used as a semiquantitative method for measuring apoptosis[34]. The culture medium was removed and centrifuged at 3000x g for 5 min to collect detached cells. 2ml of cells which is centrifuged to 3000rpm suspended in 200 µL of 1X TE Buffer and and 100 µL of 10% SDS, incubated at $60^0$ 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 500µL of Isopropanol. Add 200 µL of 70% ethanol, then centrifuge at 10,000 rpm for 10 minutes. Dry the pellet at 37 $^0$C till there are no traces of solution. Resuspend the pellet in 20 µL of 1xTE Buffer. Electrophorese the extracted DNA on 1% agarose gel. Agarose gel electrophoresis is carried out[35]. For casting 1% Agarose gel add 0.8 gm of Agarose in 80mL 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 20µL of sample DNA, mix well, and then load the total 22µL 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 DISSCUSSION:

Results of cell viability assay on normal vero cell and Hep-2 laryngeal cancer cell line are shown in (Table1&fig.1). 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 0.078mg/ml which decreased with increase in concentration. Results of anticancer activity on Hep-2 is shown in (table2). The extract showed a potent cytotoxic activity against Hep-2 laryngeal cancer cell line. Cyclophosphamide served as pc-control and 96.2% cancer inhibition was observed. The concentration of ethanolic extract of *Cynodon dactylon* at 10mg/ml showed inhibition percent with regard to cytotoxicity of 93.5% that was comparable to the positive control. Ethanolic extract of *Cynodon dactylon* at 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml, 0.156mg/ml, 0.078mg/ml showed cytotoxic activity of 90.0%, 81.6%, 74.8%, 66.1%, 59.2%, 46.9%, 32% respectively. Results of anticancer activity on HELA Cervical cancer cell line is shown in (table3&fig.1). Cyclophosphamide served as pc-control and 92% cancer inhibition was observed. The concentration of ethanolic extract of *Cynodon dactylon* at 10mg/ml showed inhibition percent with regard to cytotoxicity of 88.5% that was comparable to the positive control. Ethanolic extract of *Cynodon dactylon* at 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml, 0.156mg/ml, 0.078mg/ml showed cytotoxic activity of 86.2%, 82.9%, 73.7%, 50.2%, 42.9%, 37.6%, 27.1% respectively. Results of anticancer activity on MCF-7 Breast cancer cell line is shown in (table 4 & fig.1). The extract showed a potent cytotoxic activity against MCF-7 cancer cell line. Cyclophosphamide served as pc-control and 83% cancer inhibition was observed(table 4). The concentration of ethanolic extract of *Cynodon dactylon* at 10mg/ml showed inhibition percent with regard to cytotoxicity of 79.2% that was comparable to the positive control .Ethanolic extract of *Cynodon dactylon* at 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml, 0.156mg/ml, 0.078mg/ml showed cytotoxic activity of 68.75%, 64.6%, 52.1%, 45.9%, 39.6%, 29.2%, 14.6% 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% HEP-2 Laryngeal, HELA Cervical and MCF-7 breast cancer cells was recorded (Ic50) at a concentration of 0.156mg/ml, 0.625mg/ml of the plant extract. Among these three cancerous cell lines HEP-2 shows high activity at the concentration of 0.156mg/ml.

DNA fragmentation was obtained by agarose gel electrophoresis of ethanolic extract of *Cynodon dactylon* with Hep-2, HeLa and Mcf-7 cancer cell lines. The DNA migrated as discrete bands which was compared to DNA markers, gave a ladder of approximately 200 base pair (bp). Such DNA ladders

are considered to be a hall mark of apoptosis, continues smears may also indicate DNA fragmentation due to apoptosis. The ladder from DNA fragmentation catalyzed by an endogenous endonuclease that cleaves internucleosomal DNA to form ladder like bands of oligo nucleosome fragments. From this it is revealed that these DNA fragments (fig.3) shows that the ethanolic extract of *Cynodon dactylon* has anticancer activity in the Hep-2, HeLa and Mcf-7 cell lines.

**Table 1: Cell viability assay on Vero cell line**

| S.No | Concentration(mg/ml) | Dilution | % cell viability | Percentage of Cytotoxicity |
|---|---|---|---|---|
| 1. | Control | - | 100 | 0 |
| 2. | 0.078 | 1:64 | 97 | 3 |
| 3. | 0.156 | 1:32 | 94 | 6 |
| 4. | 0.312 | 1:16 | 92 | 8 |
| 5. | 0.625 | 1:8 | 89 | 11 |
| 6. | 1.25 | 1:4 | 86 | 14 |
| 7. | 2.5 | 1:2 | 84 | 16 |
| 8. | 5 | 1:1 | 82 | 18 |
| 9. | 10 | Neat | 79 | 21 |

**Table2: Anticancer activity of HEP2 cell line of ethanolic extract of *Cynodon dactylon.***

| S.No | Concentration(mg/ml) | Dilution | % cell viability | Percentage of cytotoxocity |
|---|---|---|---|---|
| 1. | Negative control | - | 100 | 0 |
| 2. | 0.078 | 1:64 | 68.0 | 32 |
| 3. | 0.156 | 1:32 | 53.1 | 46.9 |
| 4. | 0.312 | 1:16 | 40.8 | 59.2 |
| 5. | 0.625 | 1:8 | 33.9 | 66.1 |
| 6. | 1.25 | 1:4 | 25.2 | 74.8 |
| 7. | 2.5 | 1:2 | 18.4 | 81.6 |
| 8. | 5 | 1:1 | 9.9 | 90.0 |
| 9. | 10 | Neat | 6.5 | 93.5 |
| 10. | Positive Control | - | 3.8 | 96.2 |

**Table3: Anticancer activity of HELA cell line of ethanolic extract of *Cynodon dactylon.***

| S.No | Concentration(mg/ml) | Dilution | % cell viability | Percentage of cytotoxocity |
|---|---|---|---|---|
| 1. | Negative control | - | 100 | 0 |
| 2. | 0.078 | 1:64 | 72.9 | 27.1 |
| 3. | 0.156 | 1:32 | 62.4 | 37.6 |
| 4. | 0.312 | 1:16 | 57.1 | 42.9 |
| 5. | 0.625 | 1:8 | 49.8 | 50.2 |
| 6. | 1.25 | 1:4 | 26.3 | 73.7 |
| 7. | 2.5 | 1:2 | 17.1 | 82.9 |
| 8. | 5 | 1:1 | 13.8 | 86.2 |
| 9. | 10 | Neat | 11.5 | 88.5 |
| 10. | Positive Control | - | 8.0 | 92 |

**Table4: Anticancer activity of MCF-7 cell line of ethanolic extract of *Cynodon dactylon.***

| S.No | Concentration(mg/ml) | Dilution | % cell viability | Percentage of cytotoxocity |
|---|---|---|---|---|
| 1. | Negative control | - | 100 | 0 |
| 2. | 0.078 | 1:64 | 85.4 | 14.6 |
| 3. | 0.156 | 1:32 | 70.8 | 29.2 |
| 4. | 0.312 | 1:16 | 60.4 | 39.6 |
| 5. | 0.625 | 1:8 | 54.1 | 45.9 |
| 6. | 1.25 | 1:4 | 47.9 | 52.1 |
| 7. | 2.5 | 1:2 | 35.4 | 64.6 |
| 8. | 5 | 1:1 | 31.25 | 68.75 |
| 9. | 10 | Neat | 20.8 | 79.2 |
| 10. | Positive Control | - | 17 | 83 |

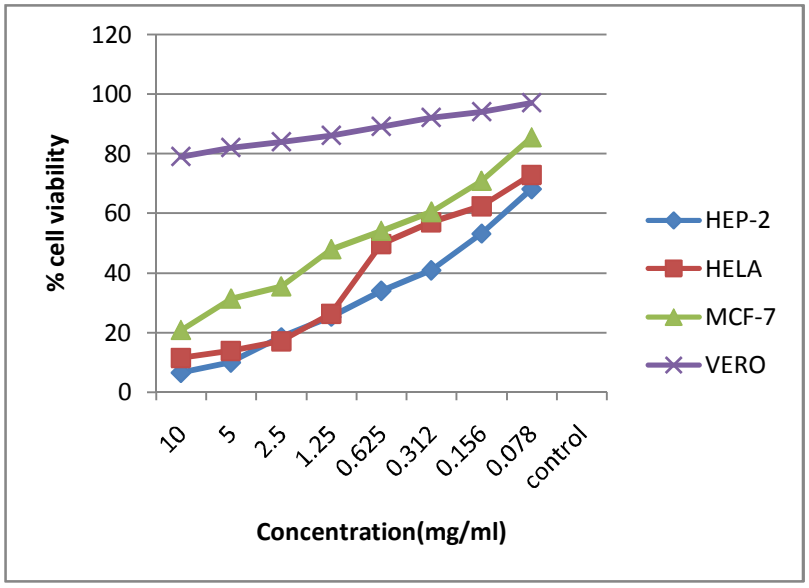
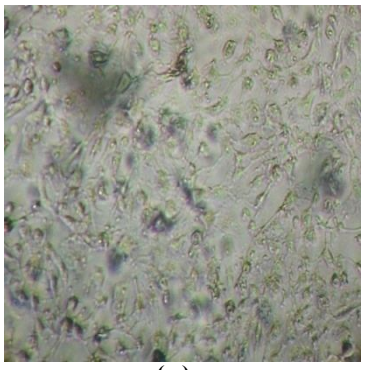
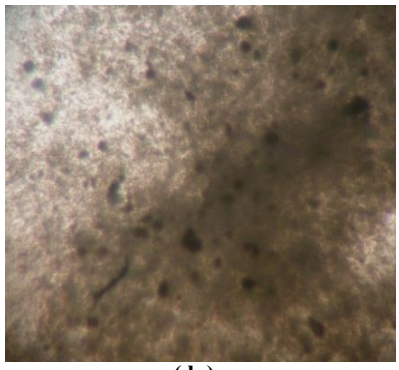


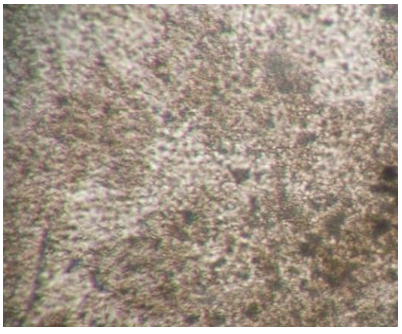

**Fig 1: Percentage of cell viability vs concentration shows that the effective drug concentration, that is non toxic to Vero cell line but toxic to Hep-2, Hela and Mcf-7 Cancer cell lines.**



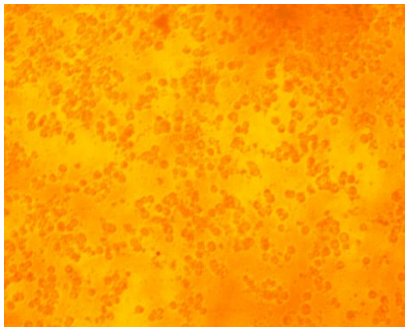
**( a)**



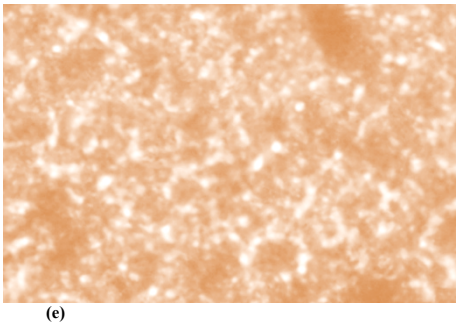
**( b )**



**( c )**



**(d)**



(e)

**Fig.2: Photomicrograph of ethanolic extract of *Cynodon dactylon* treated with cancer cell lines. a) Vero normal cell line. b) Hep-2 Cell lines treated with 0.156mg/ml of ethanol extract of *Cynodon dactylon*. c)HeLa Cell lines treated with 0.625mg/ml of ethanol extract of *Cynodon dactylon*. d) Mcf-7 Cell lines treated with 0.625mg/ml of ethanolic extract of *Cynodon dactylon.* e) Positive Control(pc).**

## *Dna fragmentation:*

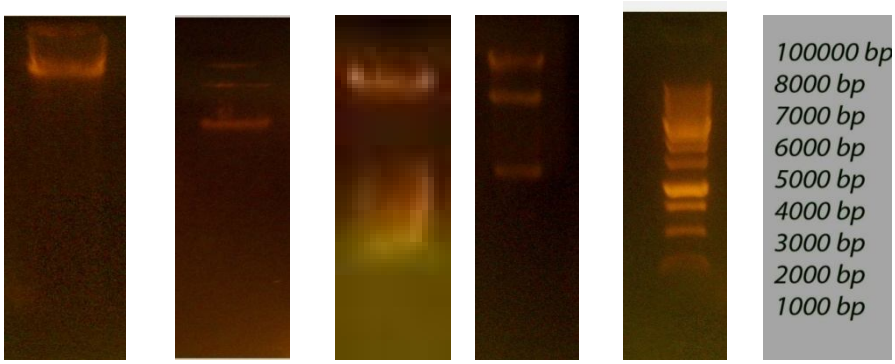



**Fig3. DNA laddering visualized in agarose gel by ethidium bromide staining of ethanolic extract of *Cynodon dactylon* a) Lane1: HEP-2 b)Lane 2: HELA c)Lane 3: MCF-7 d)Lane 4: Control e)Lane 5: kb marker**

## CONCLUSION:

The results of this study support the efficacy of *Cynodon dactylon* as an anticancer agent for Hep-2 laryngeal, HeLa Cervical and Mcf-7 Breast cancer cell lines. From the present study it has been revealed that ethanolic extract of *Cynodon dactylon* shows 50% anticancer activity in Hep-2 cancer cell line at the concentration of 0.156mg/ml, when compared to HeLa and Mcf-7 at the concentration of 0.625mg/ml. It may act as a potential adjuvant treatment to current chemotherapeutic agents and can be

used in the treatment of Hep-2, Hela and Mcf-7 cell lines. From this it is said that there may be some anticancer components, which helps for the treatment of HEP-2 laryngeal, HeLa cervical, Mcf-7 breast cancer cell lines, Further research has to be conducted for components present in the ethanolic extract of *Cynodon dactylon* which may act as the ligand and bind with these cancer cell line receptors. In future the components present on *Cynodon dactylon* may act as a drug, for the above mentioned cell lines. Further *in-vivo* studies should be carried out. Several reports describe that the anticancer activity of these plants is due to presence of antioxidants.

## REFERENCES:


1. Chung VQ, Tattersall M, Cheung HT. Interactions of a herbal combination that inhibits growth of prostate cancer cells. Cancer Chemother. Pharmacol. 2004; 53 : 384-390.
2. Cordell GA. PC-SPES: a brief overview. Integr Cancer Ther. 2002; 1: 271-286.
3. Hsieh TC, Lu X, Chea J, Wu JM. Prevention and management of prostate cancer using PC-SPES: a scientific perspective. J Nutr. 2002; 132 : 3513S-3517S.
4. Ikezoe T, Chen S, Saito T, Asou H, Kyo T, Tanosaki S, Heber D, Taguchi H, Koeffler HP: PC-SPES decreases proliferation and induces differentiation and apoptosis of human acute myeloid leukemia cells. Int J Oncol. 2003; 23 : 1203-1211.
5. Meyer JP, Gillatt DA: PC-SPES: a herbal therapy for the treatment of hormone refractory prostate cancer. Prostate Cancer Prostatic Dis. 2002; 5 : 13-15.
6. Oh WK, Kantoff PW, Weinberg V, Jones G, Rini BI, Derynck MK, Bok R, Smith MR, Bubley GJ, Rosen RT, DiPaola RS, Small EJ. Prospective, multicenter, randomized phase II trial of the herbal supplement, PC-SPES, and diethylstilbestrol in patients with androgen-independent prostate cancer. J Clin Oncol. 2004; 22 : 3705-3712.
7. K.V.K. Rao, A.S. Stanley, H.K. Nair. R. Aalinkeel, Mahajan S. Chawda, M.P.N. Nair. Plant derived products as a source of cellular growth inhibitory pytochemicals on PC-3M, DU-145 and LNCaP prostate cancer cell lines. Current Science. 2004; 87: 1585-1588.
8. G. Rosangkima and S.B. Prasad. Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dolton's lymphoma. Indian J. Exp. Biol. 2004; 42 : 981-988.
9. Chong FW, Srikumar Chakravarthi, HS Nagaraja, PM Thanikachalam, Nagarajah Lee. Expression of Transforming Growth factor-β and determination of Apoptotic Index in

histopathological sections for assessment of the effects of Apigenin (4',5',7'- trihydroxyflavone) on Cyclosporine A induced renal damage. Malaysian Journal of Pathology. 2009; 31 (1): 35–43.

10. Markovits J, Linassier C, Fossé P, Couprie J, Pierre J, Jacquemin-Sablon A, Saucier JM, Le Pecq JB, Larsen AK 1989. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res. 1989; 49 (18): 5111–7.
11. Lopez-Lazaro M, Willmore E, Austin CA. Cells lacking DNA topoisomerase II beta are resistant to genistein. J Nat Prod. 2007; 70 (5): 763–7.
12. Harlan J. *Cynodon* species and their value for grazing and Hay.Herbage Abstract. 1970; 40(3): 233-238.
13. Sujon M A, Mostofa M, Jahan M S, Das A R and Rob S. Studies on Medicinal plants against Gastrointestinal Nematodes of Goats. Bangl. J. Vet. Med. 2008; 6(2): 179–183.
14. Singh S K, Rai P K, Mehta S, Singh R K, Watal G. Curative Effect of *Cynodon dactylon* Against Stz Induced Hepatic Injury In Diabetic Rats. Indian Journal Of Clinical Biochemistry. 2009; 24(4): 410-413
15. Cheryl A L. Ethnomedicines used in Trinidad and Tobago for urinary problems and Diabetes mellitus. Journal of Ethnobiology and Ethnomedicine 2006; 45(2): 1746-4269.
16. Singh S K, Kesari A N, Gupta R K, Jaiswal D, Watal G, Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. J Ethnopharmaco. 2007; 114(2): 174-179.
17. Jarald E E, Joshi S B, Jain D C. Antidiabetic activity of aqueous extract and non polysaccharide fraction on *Cynodon dactylon* Pers. Indian journal of Experimental Biology. 2008; 46(9): 660 - 667.
18. Khajavi Rad A, Hadzadeh M A, Rajaei Z, Mohammadian N, Valiollahi S, Sonei M. The beneficial effect of *Cynodon dactylon* on ethylene glycol-induced kidney calculi in rats. Vrol J.2011; 8(3): 179 -84.
19. Das S, Dutta Choudhury M. Plants Used Against Gastro-Intestinal Disorders and As AntiHemorrhagic by Three Tribes of North Tripura District, Tripura, India. Ethnobotanical Leaflets.2010; 10(4): 467-78.
20. Kunja B. Satapathy, Binod B.Sahu, Gouri Shankar jena. Crop weeds diversity and their ethnomedicinal uses in the treatment of common ailments in Jaipur district of odisha(India). Int.J.Med.Arom.Plants. 2012; 2(1): 80 -89.

21. Oudhia P. Medicinal weeds in rice fields of Chhattisgarh (India). Int. Rice Res.1999; 24(1): 40.
22. Oudhia P. Medicinal weeds in groundnut fields of Chhattisgarh (India). Int. Arachis Newslett.1999; 19: 62-64.
23. Agharkar S P. Medicinal plants of Bombay presidency. Scientific Publ., Jodhpur, India. 1991; p. 80-87
24. Oudhia P, Joshi B S and Kosta V K. The possibilities of preparing homeopathic drugs from the obnoxious weeds of Chhattisgarh. Bhartiya Krishi Anusandhan Patrika.1998; 13(1/2):53-57
25. Dilip Kumar Pal. Determination of Brain Biogenic Amines in *Cynodon dactylon* pers & *Cyperus rotundus* treated mice. International Journal of Pharmacy and Pharmaceutical Science. 2009; 1(1): 190-197
26. Santosh Kumar Singh, Prashant Kumar Rai, Dolly Jaiswal, and Geeta Watal. Evidence based Critical Evaluation of Glycemic Potential of *Cynodon dactylon*. Evidence-based Complementary and Alternative Medicine. 2007; 5(4): 415-420
27. Saroja Marappan, Annapoorani Subramaniyan . Journal of Advanced Scientific Research. 2012; 3(1): 105-108.
28. Kaleeswaran B, Ilavenil S. and Ravikumar S. Screening of phytochemical properties and antibacterial activity of *Cynodon dactylon* L. International journal of current research. 2010; 3: 083-088.
29. Parag R. Patel, akhil A. Nagar, rikin C. Patel, dhara k. Rathod, vishal r. Patel. *Invitro*anticancer activity of *rubia cordifolia* against hela and hep2cell lines. International journal of pharmacy and pharmaceutical sciences.2011; 3(2): 0975-1491.
30. Sanjay patel, nirav gheewala, ashok suthar, anand shah. In-vitro cytotoxicity activity of *solanum nigrum* extract Against *hela* cell line and *vero* cell line. International journal of pharmacy and pharmaceutical sciences. 2009; 1(1).
31. Rahman S, Salehin F, Iqbal A. In vitro antioxidant and anticancer activity of young *Zingiber officinale* against human breast carcinoma cell lines. BMC Complement Altern_Med. 2011; 20:11-76.
32. Mosmann, Tim. Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983; 65 (1–2): 55–63.

33. Walker PR, Kokileva L, LeBlanc J, Sikorska M. Detection of the initial stages of DNA fragmentation in apoptosis. Biotechniques . 1993; 15:1032–1040
34. Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol.* 1980; 68: 251–305.
35. Kokileva L. Endogenous degradation of rat liver chromatin studied by agar gel electrophoresis of nuclei. Mol Biol Rep. 1989; 13:139–143.