

Evaluation of *in-vitro* apoptosis induction, cytotoxic activity of *Hymenodictyon excelsum* (Roxb) Wall in Dalton's lymphoma ascites (DLA) and Lung fibroblast - Mouse L929 cell lines

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ABSTRACT

The purpose of present study was to investigate the methanolic bark extract of *Hymenodictyon excelsum* (Roxb) Wall for its apoptosis, cytotoxic activities. Induction of apoptosis was carried out on L-929 cell line. The cytotoxic activity was evaluated by MTT assay against lung fibroblast (L-929) cell line and trypan blue dye exclusion assay on dalton's lymphoma ascites (DLA) cells. Morphological changes and DNA fragmentation were found upon incubation with extract. The extract was found to be cytotoxic towards L-929 cells in 72 h MTT assay and concentration required for 50% cell death was 3.85µg/ml. Thus from the present investigation it can be concluded that the methanolic bark extract of *Hymenodictyon excelsum* (Roxb) exhibited apoptosis induction and cytotoxic activities.

INTRODUCTION

The American Cancer Society estimates a total of 1,660,290 new cancer cases and 580,350 cancer deaths are projected to occur in the United States in 2013. Death rates continue to decline for all 4 major cancer sites (lung, colorectum, breast, and prostate). Over the past 10 years of data (2000-2009), the largest annual declines in death rates were for chronic myeloid leukemia (8.4%), cancers of the stomach (3.1%) and colorectum (3.0%), and non-Hodgkin lymphoma (3.0%). The reduction in overall cancer death rates since 1990 in men and 1991 in women translates to the avoidance of approximately 1.18 million deaths from cancer, with 152,900 of these deaths averted in 2009 alone (Rebecca *et al.*, 2013). Medicinal herb extracts or formulations being tested on treatments for chemo- or radiotherapies against various cancers (Shu *et al.*, 2013). *Hymenodictyon excelsum* is a deciduous tree is usually with a straight cylindrical bole and a rounded crown, having 10 to 12 meters in height.

Figure 1 shows the bark of *Hymenodictyon excelsum*. Bark is furrowed and rough (branches is smooth), 10 to 20 cm thick, grey, exfoliating in irregularly shaped, softish scales. Leaves are about 10 to 24 cm long, 7 to 12.5 cm wide and ovate-elliptic or almost rounded, pointed at both ends, and hairy on both surfaces. The flowers are stalked, white, fragrant, about 0.5 cm long, and borne in terminal, drooping panicles. The corolla-tube is slender, and 5-lobed. The fruit (capsule) is ellipsoid and 2 to 2.5 cm long, growing on recurved, thick pedicels 5 to 12 mm long (Kirtikar and Basu, 1995).

Insilico studies have shown that phytoconstituents of *Hymenodictyon excelsum* like aesculetin, aesculin, damnacnathal, morindone, nordamnacanthal, was found to inhibit gsk3beta (Karthik *et al.*, 2014). Crushed stem bark is prescribed to cure enlarged spleen of babies. Diarrhea and dysentery in babies is cured with whole plant decoction (Abhijit and Jitendranath, 2011). Methanolic bark extract of *Hymenodictyon excelsum* (Roxb.) showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans* (Chea *et al.*, 2007). The leaves were found to possess antiinflammatory activity (Winter *et al.*, 1962).

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The search for anti-cancer drugs from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vincristine and vinblastine, and the isolation of the cytotoxic podophyllotoxines.



Fig. 1: The diagram showing the bark of *Hymenodictyon excelsum*.

These discoveries prompted the United States National Cancer Institute (NCI) to initiate an extensive plant collection program in 1960, focused mainly in temperate regions. A frequent liability of natural products, at least in the area of cancer chemotherapy, is that, although many are generally very potent, they have limited solubility in aqueous solvents and exhibit narrow therapeutic indices. These factors have reported in the demise of a number of pure natural products, such as the plant-derived agents, bruceantin and maytansine, as promising leads. An another approach to utilizing such agents is to investigate their potential as ‘warheads’ attached to monoclonal antibodies specifically targeted to epitopes on tumors of interest (Samy *et al.*, 2008).

Plants have a long history of use in the management of cancer. Hartwell, in his review of plants used against cancer, lists more than 3000 plant species that have reportedly been used in the treatment of cancer (Cragg *et al.*, 1994). Chemotherapy is one of the promising methods for cancer control. Studies and interests in cancer chemoprevention by the biological activity and pharmaceutical value of naturally occurring substances, which were derived from food and medicinal plants, have increased in recent decades.

In particular, the discovery of natural products with specific action on tumor cells would be helpful in cancer chemoprevention or chemotherapy (Kametani *et al.*, 2007). We have selected *Hymenodictyon excelsum* on the basis of its chemical constituents (diverse flavonoids, anthraquinone derivatives) as a single moiety showed various anti-apoptotic activities.

H. excelsum containing all those phytoconstituents would have some cytotoxic activity. On this basis, investigation on extract of bark of *H. excelsum* has been made on using novel *invitro* screening methodologies using cell lines.

MATERIALS AND METHODS

Dalton’s lymphoma ascites (DLA) cell lines and Lung fibroblast - Mouse L929 cell lines were purchased from amla cancer research institute, Thrissur, kerala. Phosphate buffered saline, streptomycin, Benzyl penicillin and DMEM medium from himedia Laboratories, Mumbai. 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide, RNA-ase, proteinase k, curcumin, ambistyrin, NTE buffer, trypsin, sodium dodecyl sulphate, sodium acetate, TAE buffer, ethidium bromide, Trypan blue were purchased from Sigma Aldrich, St. Louis, USA.

Plant material

The plant material consists of dried powdered bark of *Hymenodictyon excelsum* (Roxb) Wall belonging to the family Rubiaceae (Kirtikar and Basu, 1995).

Collection and authentication

Stem bark of *Hymenodictyon excelsum* (Roxb) Wall was collected from Thrissur district, Kerala, India and during the month of June 2013. The plant material was identified and authenticated by Mr. G. V. S Murthy, Joint Director, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SRC/5/23/12-13/Tech-657.

Extraction procedure for *Hymenodictyon excelsum* (Roxb) Wall

The fresh bark of *Hymenodictyon excelsum* (Roxb) Wall was collected. The collected bark was dried in shade under room temperature, powdered mechanically and sieved through No. 20 mesh sieve.

The finely powdered bark was kept in airtight container until the time of use. The Fresh stem bark (100g) was exhaustively extracted with 95% MeOH (250 ml) using Soxhlet apparatus. It was filtered and the filtrate condensed under reduced pressure. The extract was concentrated to dryness under controlled temperature 40-50°C. The percentage yield of *Hymenodictyon excelsum* (Roxb) Wall bark extract (HEBE) was 8.55%w/w (Raad *et al.*, 2013)

Phytochemical screening

Chemical test were carried out for the bark of *Hymenodictyon excelsum* (Roxb.) Wall. for the presence of phytochemical constituents (Trease and Evans, 2002;Kokate CK, 1994).

Cytotoxicity Screening

Cell lines

Dalton’s lymphoma ascites (DLA) cells (Raju *et al.*, 2013) were used for short term *in vitro* cytotoxicity experiments. This cell lines were maintained as ascites tumors in swiss albino mice. The cells were aspirated, washed thrice in normal saline counted using a haemocytometer and cell suspension of 1million

cells/ml was prepared. One ml of this suspension was injected into peritoneal cavity of swiss albino mice. Lung fibroblast - Mouse L929 cell lines (Harhaji *et al.*, 2009) were used for long term *in vitro* cytotoxicity experiments.

Short term *in vitro* cytotoxicity assay by trypan blue dye exclusion technique

Any compound, which is cytotoxic to cells, inhibits the cell proliferation and kills the cells. Trypan blue has the ability to penetrate in to the dead cells and give it blue color. This method gives an exact number of dead and viable cells (Kuttan *et al.*, 1985).

Cells were aspirated from the peritoneal cavity of tumor bearing mice and it was washed three times using PBS. The viability of cells were checked using trypan blue (cell viability should be above 98%).

The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures was incubated for 3h at 37°C and then 1ml of trypan blue was added after incubation and the number of dead cell was counted using a haemocytometer (Shrivastava and Ganesh., 2010). The percentage cytotoxicity was calculated using the equation 1 shown below.

$$\% \text{ cytotoxicity} = \left[\frac{\text{No. of dead cells}}{\text{No. of viable cell} + \text{No. of dead cell}} \right] \times 100 \quad \dots \text{eqn (1)}$$

Long term *in vitro* cytotoxicity by MTT assay

The ability of the cells to survive a toxic insult is the basis of most cytotoxic assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium.

The assay depends both on the mitochondrial activity per cell and number of cells present. The cleavage of 3-(4, 5 dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based. The principle involved is the cleavage of tetrazolium salt, 3-(4, 5 dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) in to a blue colored product (formazan) by mitochondrial enzyme succinate dehydrogenase. The amount of cells was found to be proportional to the extent of formazan production by the cells used (Ramnath *et al.*, 2009).

Cells were seeded in a 96-well flat-bottom plate (5000 cells/well) and permitted to adhere for 24h at 37°C with 5% CO₂ atmosphere. Different drug concentration was added and incubated further for 48hrs. Before 4h of the completion of incubation, 20µl of MTT (5mg/ml) was added. Dead cell percentage was determined using an ELISA plate reader set to record absorbance at 570nm. The percentage growth inhibition was calculated using the formula given below (Hajighasemi and Mirshafiey., 2010).

$$\% \text{ Growth inhibition} = 100 - \left[\frac{\text{OD of individual test group}}{\text{OD of Control Group}} \right] \times 100. \text{eqn (2)}$$

Apoptosis detection assay

Apoptosis is the mechanism by which the cells undergo programmed cell death. It is a physiological program characterized by chromatin condensation, membrane blebbing, cell shrinkage and DNA fragmentation (Sharma *et al.*, 2007).

DNA ladder analysis

DMEM medium (912mg/100ml) containing benzyl penicillin 1 mg/litre, Ambistyrine (1mg/litre) was prepared, adjust the pH to 7.0 with sodium bicarbonate. To the medium add 2 X 10⁶ DLA cells (suspended in minimal volume), 2µl trypsin and different drug concentration and then make the final volume to 2ml and incubate at 37 °C for various hours (from 1-24 hrs). After incubation centrifuge the cells at 10,000 rpm for 10mins, discard the medium and wash the cells for 2 times in NTE buffer. Suspend the cells in 2ml NTE buffer and 2 % trypsin (100µg/ml) and add 20 % SDS (25 µl/ml) and proteinase K (100µg/ml). Incubate the cells at 37 °C for overnight. Add 1ml NTE buffer saturated phenol and 1ml chloroform and shake the vial 12 times (turning up and down slowly) centrifuge at 10,000 rpm for 10 min (2 phases are seen) and transfer the upper portion to another vial and add 1ml chloroform, repeat this for 4 times. To this RNase was added and incubated at 35 °C for 2hrs. Centrifuge the vial at 10,000rpm for 10mins and decant the solvent and take the pellet (DNA) and dissolve in TAE buffer. Then the dissolved DNA is subjected to horizontal electrophoresis (Lin *et al.*, 2009)

Morphological studies

To detect the morphological changes during apoptosis, 5x10⁶ DLA cells were incubated in DMEM at 37°C in the presence of 5% CO₂ for 48 hrs. After incubation, the cells were washed twice with PBS, centrifuged and the cell pellets were smeared on a clean glass slide. The slides were fixed in methanol and stained with hematoxylin-eosin method. The slides were observed at 100X for the changes (Zahri *et al.*, 2009).

RESULTS

Cytotoxicity studies

Short term *in vitro* cytotoxicity studies by trypan blue dye exclusion method

Cytotoxic activity of *Hymenodictyon excelsum* (Roxb.) Wall bark extract on DLA cells was evaluated by Trypan blue dye exclusion method. Cytotoxicity of HEBE to DLA cell culture is shown in Table 1.

Haemocytometer were counted the viable cells which remained unstained by trypan blue. Table-1 represents the percentage cytotoxicity of the DLA cells at different concentrations ranging from 10µg/ml to 200µg/ml. Extract showed the viable cells which remained unstained by trypan blue were counted with the use of haemocytometer. Concentration needed

Table 1: Cytotoxicity screening of HEBE against DLA cells lines.

Sample	Concentration (µg/ml)	Percentage cytotoxicity	CTC ₅₀
Control		1.10±2.53	
HEBE	10	27.34±0.19	18.50±2.22 *
	20	54.62±0.93	
	50	82.61±1.24	
	100	99.99±1.81	
	200	100.00±1.61	
Curcumin	10	39.23±1.02	14.75±3.51*
	20	63.21±2.28	
	50	85.24±2.88	
	100	100.00±1.06	
	200	100.00±1.32	

Values are mean± S.D, n=3 *P< 0.01 when compared with control.

Table 2: Long term cytotoxicity screening of HEBE against L929 cell lines by MTT assay.

Sample	Concentration (µg/ml)	Absorbance at 570 nm	Percentage cytotoxicity	CTC ₅₀
Control		0.405±0.01		
HEBE	2	0.285±0.012	29.63±0.61	4.56±0.200
	5	0.146±0.045	63.95±0.10	
	10	0.108±0.023	73.34±0.26	
	15	0.020±0.012	95.06±0.41	
	20	0.001±0.001	97.53±0.88	
Curcumin	2	0.201±0.23	50.37±0.15	1.70±0.010
	5	0.132±0.19	67.41±0.23	
	10	0.045±0.27	88.89±0.27	
	15	0.000±0.00	100±0.97	
	20	0.000±0.00	100±0.39	

Values are mean± S.D, n=3 *P< 0.01 when compared with control.

for 50% inhibition of growth of DLA cells was found to be 18.50 µg/ml. Curcumin was used as the reference drug and it produced 100% cytotoxicity at 100 µg/ml and 200 µg/ml and CTC₅₀ value obtained was 14.75 µg/ml. Compared to standard HEBE was found to have cytotoxic effects. This result emphasized cytotoxic nature of HEBE against DLA cells, which was dose dependent and were statistically significant with P< 0.01.

Long term in vitro cytotoxicity studies by MTT assay

Cytotoxic effect on L929 cells was investigated by MTT assay for the relationship between concentration of HEBE and the results were given in table 2. Cells were treated with HEBE at concentrations ranging from 2-20 µg/ml for 48 h and then the percentage of cell viability was analysed. HEBE is significantly (P< 0.01) inhibited the proliferation of L929 cells in a dose dependent manner. The CTC₅₀ of the HEBE was found to be 4.56 µg/ml on L929 cell lines. The CTC₅₀ value of the curcumin (reference drug) was found to be 1.70 µg/ml. HEBE showed a concentration dependent cytotoxicity to cultured L929 cells that was almost comparable with that of curcumin and were statistically significant with P < 0.01.

Apoptosis detection assay

Morphological analysis of apoptotic cells

Microscopical examination of HEBE treated L929 cells (Fig. 2) revealed that the cell death is due to apoptotic characteristics. It shows characteristic morphology when a cell undergoing apoptosis. Apoptotic cells were distinguished as highly shrunken cell, nuclear condensation, fragmentation, margination, cell blebbing and presence of apoptotic bodies.

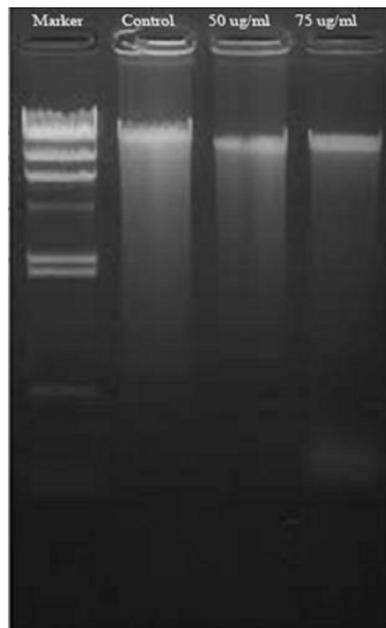


Fig. 3: Showing the DNA fragmentation with respective concentration of extracts of HEBE Lane 1: DNA molecular weight marker ; Lane 2: DNA isolated from fresh L929 cells ; Lane 3: DNA isolated from HEBE treated (50µg/ml) L929 cells ; Lane 4: DNA isolated f.

Fig. 3 showed the result of horizontal electrophoretic run of HEBE treated L929 cells exhibited extensive double strand breaks; thereby yielding a ladder appearance was shown in lane 4, while the DNA isolated from fresh L929 cells did not show any double strand breaks in lane 2. The DNA isolated from L929 cells treated with 75µg/ml HEBE showed fragmentation.

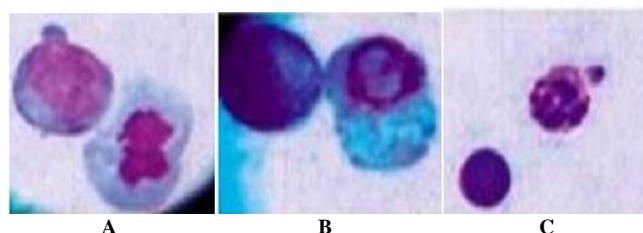


Fig. 2: Morphological analysis of apoptotic cells (a) showing cells begin to shrink and because of breakdown of proteinaceous cytoskeleton rounding are shown. (b) and (c) of HEBE showing chromatin condensation and cell blebbing of the nuclear membrane.).

DISCUSSION

The majority of the cancer treatments are accompanied by a degree of herbal supplements. There are advantageous effects of medicinal plants on cancer. Several therapies include herbal remedies to improve the quality of life for the sufferer as well and aid the potent medicine to do its job of ridding the body of this

fatal disease. Around the world there are countless trees, herbs and fruits that possess anti-cancer properties. Cancer patients becoming are more knowledgeable about this matter by learning about the positive effects of medicinal plants on cancer and finding their own success by taking high quality supplements to beat the disease (Spring, 2010).

Plant derived natural products such as flavonoids, terpenes, alkaloids etc. have received wide attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Babu *et al.*, 2002). The look for anti-cancer agents from plant sources started in the 1950s with the discovery and development of the vinca alkaloids like vincristine and vinblastine, and the isolation of the cytotoxic podophyllotoxines. These discoveries encouraged the United States National Cancer Institute (NCI) to initiate an extensive plant collection programmes in 1960, focused mainly in temperate regions. This led to the discovery of a lot of novel chemotypes showing a range of cytotoxic activities, including the camptothecins and taxanes (Cragg and Newman., 2005).

Natural products discovered from medicinal plants have played a vital role in the management of cancer. Natural products or natural product derivatives consist of 14 of top 35 drugs in 2000 based on worldwide sales (Butlet MS., 2004). Plant based medication has definitely found a role in cancer healing (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively. In particular, there is growing interest in the pharmacological estimation of various plants used in Indian tradition system of medicine. There are more than 2,70,000 higher plants existing on this planet. But only a small portion has been surveyed phytochemically. So, it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat cancer diseases (Shoeb M., 2006).

The phytochemical screening of HEBE showed presence of chemical compounds such as alkaloids, flavonoids, glycosides, phenolics, steroids, tannins and terpenoids, whereas saponin is absent in HEBE. Medicinal plants may contain various kinds of chemical components and their biological activities are not generally attributable to a single moiety (Cho *et al.*, 2003). The present study was undertaken to assess the cytotoxic activity of HEBE and AGSE. The cytotoxic result obtained in the present study demonstrates for the first time, to the best of our knowledge, that HEBE and AGSE caused a dose-dependent growth inhibitory effect.

Chemoprevention, which consists of the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings, is an extremely promising strategy for cancer prevention. The control of cell proliferation is fundamental in maintaining cellular homeostasis and loss of this mechanism is a principle hallmark of cancer cells. Thus the inhibition of tumor cell growth without side effects is recognized as an important target for cancer therapy (Koppikar *et al.*, 2010).

In this study, methanolic bark extract of *Hymenodictyon excelsum* (Roxb) Wall was used. As it is known that different cell

lines might display different sensitivities towards a cytotoxic compound, the use of more than one cell line is thus considered necessary in the detection of cytotoxic compounds (kamuhabwa *et al.*, 2000). Bearing this in mind, two cell lines of different histological origin were used in the present study. Cytotoxic specificity of plant extracts is expected to be due to the occurrence of different classes of compounds in the extract, as it has been documented in the case of known classes of compounds (Cragg *et al.*, 1994).

In vitro confirmation of the extract's toxicity was done on DLA cell lines. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique. Trypan blue is an acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan blue is an essential dye, use in estimating the number of viable cells present in a population (Patel, 2009). In this study HEBE and AGSE inhibits growth in DLA in a dose dependent manner. The results of the trypan blue dye exclusion assay indicated that HEBE and AGSE extracts could inhibit the growth of DLA cells significantly ($P < 0.01$) in culture .

The cytotoxic activity was carried out by using standard MTT assay. This colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. The ability of the cells to survive a toxic insult is the basis of most cytotoxic assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the mitochondrial activity per cell and number of cells present. The cleavage of 3-(4, 5 dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based.

The reduction of MTT can only take place in metabolically active cells and the level of activity is a measure of the viability of the cells (Wilson and John, 2000). The amount of cells was found to be proportional to the extent of formazan production by the cells used. MTT reduction as a cell viability measurement is now extensively chosen as the most advantageous end point (Wahab *et al.*, 2009). This result showed that natural compounds present in HEBE and AGSE significantly ($P < 0.01$) inhibited the proliferation of L929 cells in a dose dependent manner.

These cancer effects were further studied using morphological assessment of cancer cells using agarose gel electrophoresis (Crutchen and Broek., 2002). Anti-cancer agents can modulate programmed cell death (apoptosis), may be able to affect the steady state of cell populations that are helpful in the management and therapy of cancer. Cells are dies in two ways: death by injury and death by suicide. The pattern of events in death by suicide is called apoptosis (Adams JM *et al.*, 2003).

Programmed cell death is needed whenever tissue modelling is required, during embryogenesis, for the removal of

old cells and to prevent overgrowth after repair of cell loss by injury (Kerr *et al.*, 1972). Programmed cell death is also needed to destroy cells that represent a threat to the integrity of the organism, such as virus-infected cells, effectors cells of the immune system that are no longer necessary and cells with DNA damage that can become cancerous. The mechanism of apoptosis is switched on by the imbalance between proapoptotic (death signals) and antiapoptotic (survival signals) factors. This happens in both normal and cancer cells. Dysregulation of apoptosis occurs in cancer cells (Marsoni and Damia *et al.*, 2004). In our study, HEBE cause cell growth inhibition and induce apoptosis differentially in cancer cells. Apoptosis is a well-known biological response expressed by cells after suffering DNA damage and is a useful marker for screening compounds for subsequent development as possible anticancer agent (Arulvasu *et al.*, 2010). Most of the cytotoxic antitumor drugs in recent use have been shown to induce apoptosis in susceptible cells. It has been established that apoptotic cells exhibited DNA fragmentation at inter-nucleosomal sites followed by morphological changes and loss of membrane integrity (Fan *et al.*, 2005). Further studies confirmed that, the cytotoxic potential of HEBE is closely associated with chromatin condensation, one of the well markers for apoptosis. The loss of chromatin integrity is often induced by activated caspases. It could be established that nuclear changes as a part of the apoptosis are followed by the loss of membrane integrity there by making trypan blue permeable. This study revealed that the potency of HEBE and AGSE to bring about the morphological changes like cell shrinkage, compaction and segregation of the nuclear chromatin, with the result of chromatin margination and condensation of the cytoplasm. Development of the condensation is accompanied by convolution of the nuclear and cell outlines followed by breaking up of the nucleus into discrete fragments and by budding of the cell as a whole to produce membrane-bounded apoptotic bodies (Merlin *et al.*, 2010). In addition, apoptotic characteristic DNA strand-breaks were observed by means of gel electrophoresis (Chen and Goeddel., 2002). This induction of apoptosis in cancer cells that make them more render for host phagocytic clearance without initiating inflammation, could be attributed for the extracts tumoricidal activity.

CONCLUSION

In conclusion, there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived from plants in the recent years. Based on the evaluation done using the various *in vitro* assay models it may be concluded that *Hymenodictyon excelsum* (Roxb.) Wall possess anticancer activities. Methanolic extracts of bark of *Hymenodictyon excelsum* (Roxb.) Wall is moderately active for the treatment of cancer. Further pharmacological study using other cancer cells is necessary in order to establish whether these plants can be used as a potential source for new anticancer medicine. Therefore it is suggested that further work could be done on the identification and isolation of the active constituents

and the exact mechanism responsible for the particular activity should be explored.

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