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Original Article

Evaluation of Anti-proliferative and Antioxidant Potency of *Ficus benghalensis* Hydroalcoholic Bark Extract against Lung Cancer Cell Line-A549

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ABSTRACT

Background and Aim: One of the most prevalent cancers worldwide is lung cancer with the second top fatality rate. The purpose of this work is to disclose the anti-proliferative ability of hydroalcoholic *Ficus benghalensis* bark extract against lung cancer cell line (A549). **Materials and Methods:** Antioxidant property of *Ficus benghalensis* bark extract was studied using α, α-diphenyl-β-picrylhydrazyl and Hydrogen Peroxide assays. The investigation of anti-proliferative property of *Ficus benghalensis* hydroalcoholic bark extract was carried out through tetrazolium salt-based cytotoxicity assay, cell viability assessment using trypan blue dye and morphometric analysis. The cytotoxic potency was analysed by propidium iodide staining, oxidative stress markers determination and expression of apoptotic gene markers such as Bax, c-MYC and PARP. **Results:** The anti-oxidant assays revealed the great radical scavenging property of *Ficus benghalensis* bark extract. *Ficus benghalensis* hydroalcoholic bark extract showed 50.12% inhibition at 50 µg/mL which is also confirmed by viability assay. In morphometric analysis the distortion of cells were noted in treated groups. The nuclear staining and gene expression studies further confirms the anti-cancer activity of Ficus *benghalensis* hydroalcoholic bark extract. The estimation of release of nitric oxide and lipid peroxidation was high in treated groups which also supports the previous results. **Conclusion:** The study emphasize the anti proliferative and anti oxidant property of *Ficus benghalensis* hydroalcoholic bark extract against A549 cell line.

Keywords: *Ficus benghalensis* bark extract, Lung cancer cell line, Anti proliferation, Apoptosis, Oxidative stress, Anti-oxidant.

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INTRODUCTION

One of the more dangerous cancers with a high fatality rate is lung cancer[1] in both men and women around the globe. The occurrence of cancer is due to many reasons such as smoking, dusty environment, air pollution, heredity, etc.,[2] Many chemotherapeutic drugs are available in market which creates terrible side effects and painful consequences to patients. Though, it treats cancer at some extent; there was some limitations such as each drug require expertise in biomolecular diagnostics to assure the efficacy of treatment and most of the drugs were under clinical trial.[3] So, to overcome the circumstances we need to emerge the holistic of herbal plant which possess anti-cancer property and rich antioxidant property. Many plants have been proven for its effective anti-cancer property for various types of cancer. Plant's constituents such as polyphenols, flavonoids, terpenoids, alkaloids, etc., has are markable therapeutic feature against cancer cell lines.[4] Polyphenols from the plant-based foods plays a major role in preventing cancer via eliminating carcinogen from body through cell signal alterations in cancer cells, elevation of antioxidant enzymes and arresting the cell cycle in cancer cells. Similarly, flavonoids are also effective in prevention of cancer through inhibiting signal transmitting enzymes which regulates cell differentiation and proliferation such as Protein Tyrosine Kinase (PTK), Phosphoinositide 3-Kinases (PIP3) and Protein Kinase C (PKC).[5]

Ficus benghalensis, a fig tree in the Moraceae family (common name-banyan tree), which is endemic to Bangladesh and India. *Ficus* plants possess high nutrient profile and rich polyphenols. All parts of banyan tree such as fruit, seed, leaves, stem and bark have high antioxidant and immunomodulatory properties, because of its superior qualities. Due to its therapeutic properties against inflammation, tumour, ulcer, pyretic, diabetic, bacterial and fungal infections and epileptic etc., *F. benghalensis* has been included in several traditional herbal medicines for centuries.[6] Notably, *Ficus benghalensis* bark has immense phytoconstituents profile. Several active compounds such as anthocyanidin

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derivatives, beta sitosterol-alpha-D-glucose, mesoinsitol and aliphatic long chain ketones found in the bark of *F. benghalensis*.[7-9]

Earlier research reveals that *F. benghalensis* bark extracts have potential effects to prevent or cure inflammation, diabetic, hypolipidemic, helminthic, allergic, wounds, leucorrhoea, lumbago, ulcers pain and stress etc.,[10,11] *F.benghalensis* ariel roots, latex, leaf extracts subjected to anti-cancer analysis against HepG-2, MCF 7, HeLa, MDAMB, WEHI-164 and A549 cell lines at initial level. Though, bark extract has rich phytochemical profile, there was very limited attempts in lung cancer cell line (A549). The studies only showed the initial level of screening. The present study is designed based on these contexts.

In this circumstance, we disclose the anti-cancer property of *F. benghalensis* Hydroalcoholic Bark Extract (FBHBE) against the life-threatening lung cancer via A549 cell line with various parameters. FBHBE, its anti-cancer potency was proven with cytotoxicity analysis, cell viability by trypan blue exclusion assay, analysis of various oxidative stress markers and study the gene expression level.

MATERIALS AND METHODS

Plant extract preparation

Ficus benghalensis bark was collected and washed twice with distilled water to remove the dirt adhered. Then, the bark was dried and powdered. The powdered bark was weighed and mixed with Ethanol: water (70:30). Stirred overnight or 24 hr to extract the water soluble and alcohol soluble phytoconstituents. Then, the precipitate was filtered through Whatman filter paper No. 1 (Sigma) and kept for drying at 50°C for 24 hr. The dried powder was weighed and stored for further studies.[12]

Antioxidant activity of FBHBE

The radical scavenging activity of FBHBE was assessed using stable free radical DPPH and by hydrogen peroxide assay. Vitamin C used as a standard scavenger. DPPH assay was performed according to Chowdhary *et al.*, 2014[13] with slight modification. Equal volume of various concentrations of FBHBE and DPPH (0.1 M in methanol) was taken and incubated 30 min in dark. The optical density was recorded at 517 nm (A1). DPPH with methanol was kept as blank (A0). In hydrogen peroxide assay, 40 mM hydrogen peroxide was prepared. 1 mL of various concentration of FBHBE was taken with 0.6 mL of H_2O_2 solution and kept for 10 min. The absorbance was recorded (A1) against blank (A0-H_2O_2 solution alone) at 230 nm.[14] The 50% of inhibition was calculated as ((sample-blank) /blank*100).

Cell line and cell culture

Lung cancer cell line (A549) was bought from ATCC, USA. $5x10^4$ cells were cultured in T25 flask using 10% DMEM media. Cells were incubated at 37°C for 48-72 hr up to 70-80% confluency. Cells were sub-cultured for the further studies.

Cytotoxicity analysis by MTT assay

The viability percentage of cells was determined by MTT assay method after treatment with FBHBE sample.[15] Shortly, $5x10^3$ cells/well were grown in 96 well plate in controlled conditions for 48 hr. The media was aspirated and added fresh 5% media with different concentration of FBHBE (100 µg/mL-0.1 µg/mL) and incubated for 24 hr. The effect of FBHBE was observed with microscope. Then, the media was replaced with 15 µL/well of 0.5 % MTT prepared with PBS. Plates were incubated for 4 hr in dark for the reduction of MTT. The reduced product was dissolved using 200 µl dimethyl sulfoxide and left for 10 min. The intensity of purple color was read at 490 and 630 nm in microtiter plate reader (EL×800, BioTek Instruments, Inc.). The experiment was triplicated, and untreated cells were kept as control. The percentage inhibition was calculated as follows,

$$\% \text{ inhibition} = [1- \{(OD \text{ treated}_{490}\text{-}OD \text{ treated}_{630})\text{-}(OD \text{ control}_{490}\text{-}OD \text{ control}_{630})\}X\ 100]$$

Trypan blue exclusion (TBE) assay

Viability percentage of cells was studied using TBE assay performed by Yang *et al.*, 2000[16] with slight alterations. $1x10^4$ cells were seeded in 24 well plates and incubated at appropriate condition for cell adherence and growth for 24 hr. Then, the media containing the fixed dose ranges of FBHBE was added and incubated for 24 hr. Then, the cells were trypsinized and collected by centrifugation. Trypan blue dye was added to the cell pellet, and proceeded to count the number of cells using hemocytometer through phase contrast microscopy. The number of live and dead cells were counted and recorded. The count was compared with the control or untreated cells and graph was plotted accordingly.

Morphometric analysis

$1x10^4$ cells were grown in 24 well plates for 24-48 hr. The fixed concentrations of FBHBE were added to each well with media after 24-48 hr of incubation. The treated plates were kept for 24 hr whereas untreated wells kept as control. The cells washed with PBS buffer once and morphological structure was observed under phase contrast microscopy. The images photographed using (Optika, Italy).[17]

Fluorescence staining

The apoptosis of lung cancer cell line was confirmed by fluorescence staining method using Propidium Iodide as the method followed by Moongkarndi *et al.*, 2004[17] with slight modification. $1x10^4$ cells were seeded in 24 well plates and incubated up to 70% confluency. Then, various concentrations of FBHBE were added to each well and incubated for 24 hr. The media was aspirated, and cells were washed with PBS for two times and fixed with Methanol or 4% formaldehyde for 10 min. The plates kept for air dried. PI stain 50 µg/ mL was added and kept incubated for 30 min in dark

followed by observed in Fluorescence microscope. The images were photographed.

Nitric oxide estimation

Nitric oxide was estimated in treated and untreated A549 cell line as the method followed by Yaraee *et al*., 2011[18] with slight changes. 1% Sulphanilamide solution (Griess A) and 0.1% NED solution (Griess B) were allowed to equilibrate at room temperature (15-30 min) and prepared with 5% Phosphoric acid. 100 µL of each culture supernatant treated and untreated were added to 96 well plates in triplicates. 50 µL of Griess A was added to the culture supernatant, followed by 50 µL of Griess B was added and incubated for 5-10 min in dark. Then, the plate was taken into plate reader and read at 543 nm. The optical density values obtained for treated and untreated groups were compared with the standard curve and calculated. The NO release was expressed in µM.

Estimation of Lipid peroxidation

Fluorescent complex formed between the Malonaldehyde molecule and thiobarbituric acid was determined spectrophotometrically to determine the lipid peroxidation activity with little modification of method followed by Ohkawa *et al*., 1979.[19] Treated and untreated cell culture supernatants were mixed with 750 µL of 20% acetic acid and 0.8% TBA. The total content was kept at 90°C for 1 hr then chilled with ice for 10 min. The OD was taken at 532 nm and recorded. The standard curve was plotted using MDA and concentration was calculated from the graph. The LPO release was expressed in µM.

Analysis of apoptosis-Gene expression analysis

Apoptosis related genes were assessed in treated and untreated A549 cell lines. Briefly, the cells were seeded and grown up to 80-90% confluency. The cells were treated with FBHBE with various concentrations and untreated kept as control for 24 hr. The cells were collected by trypsinization, and the cells were subjected to DNA isolation using Phenol-Chloroform extraction method.[20] Cells were incubated 16-18 hr with digestion buffer at 50-55°C followed by addition of equal volume of Phenol extraction buffer. The contents were centrifuged, and the top aqueous layer was collected in fresh tubes after centrifugation. To the collected layer, based on the volume obtained, ethanol (twice) and 0.5 M ammonium acetate (half) was added centrifuged and aspirated the supernatant. 70% ethanol (1 mL) was added to the pellet and centrifuged followed by air dried and diluted with 1X TE buffer. The DNA was used as template for PCR reactions. PCR was performed using optimized protocol with Bax, c-MYC and PARP genes with the house keeping gene Beta actin. The primer sequences are as follows (Table 1).

Statistical Analysis

Data of all the experiments were presented as Mean±SD. Results were analyzed by GraphPad prism in one-way ANOVA. Tukey's multiple comparison test used to compare the groups where $p<0.05$ consider as significant.

RESULTS

Radical scavenging analysis

Antioxidant property of FBHBE was investigated by radical scavenging assays. The balancing of free electrons known as radical scavenging activity. DPPH and hydrogen peroxide assays were used to measure the radical scavenging activity towards the free electrons. Table 2 denotes that the radical scavenging activity of FBHBE, which showed 49.42±0.48 and 48.61±0.07 for DPPH and H_2O_2 method, respectively. These results compared with the standard scavenger i.e., Vitamin C showed 50.67±0.79 and 46.95±0.11 for both assays, respectively. The results indicated that the FBHBE possesses high antioxidant property.

Cytotoxicity analysis

Cytotoxic evalution of FBHBE was performed and the obtained results was given in Figure 1. The graph showed the inhibition percentage based on the concentration of extract. From the graph, it is clear that the extract possesses noticable cytotoxic impact against lung cancer cell line. There was significant reduction was noted in proliferation with respect to increase in concentration. 50 µg/mL of extract showed 50.127% inhibition, which is fixed as IC_{50} value of FBHBE for A549 cell line.

Trypan blue exclusion assay

The assessment of cell viability was performed for the further confirmation of cytotoxicity evaluation. The result obtained was in lined with the MTT result and confirms the anti-proliferation property of FBHBE. Trypan blue assay result was denoted in Figure 2. The Figure 2 showed the reduction of cell count in dose dependent manner. 50 µg/mL of extract showed 49.88% of cell viability, which is very much simillar to the MTT assay result. Notably, a low concentration of extract at 10 µg/mL had a significant effect on cell viability, with 70% viability. The low dose alone demonstrated strong anti-proliferative activity, with a 30% inhibition.

Morphological analysis

Morphological changes of treated and untreated samples were observed using Phase contrast microscopy showed in Figure 3. The image showed there was healthy cells observed in control group, whereas the cell morphology detoriation was noticed as dose dependent manner. At middle and high dosage such as 50 and 75 µg/mL, the increased count of dead cells were seen as dose dependent manner. In low dose, i.e. 25 µg/mL, there was very less percentage of dead cells were found compared to the control.

Fluorescence analysis

The apoptosis was further confirmed by fluorescence assay. PI stain binds with nuclei of cells by which the status of nuclei can be evaluated. The treated and untreated cells were subjected to PI staining method. The results depicted in Figure 4. The Figure 4 showed that there were healthy cells with distinct nuclei was observed in control group whereas the condensed nuclei and increased number of apoptotic bodies were found in treated groups in dose dependent manner. In 75 µg/mL, there was enormous number of condensed nuclei and apoptotic bodies were observed than compared to 25 and 50 µg/mL. The resulted images of PI staining supports the previous assays results.

Nitric oxide assay

Nitric oxide release was analyzed with the treated and untreated group of cells to determine the oxidative stress level undergone by the cancer cells in the pathway of apoptosis. NO estimation was done by Griess method and the results were depicted as graph showed in Figure 5. The NO graphical image denotes that there was significant increase in the release of Nitric Oxide level in the treated group whereas control group showed less than 5 µM/mL of NO release. In 50 and 75 µg/mL treated groups, it showed almost 80 µM/mL and 100 µM/mL respectively which indicates the cell cycle arrest in A549 cell line upon treated with FBHBE.

Lipid peroxidation assay

Lipid peroxidation assays are used to determine the efficacy of antioxidants and assess oxidative damage in cells during pathophysiological conditions. The estimation of LPO was achieved using TBA method. The release of LPO was determined with help of MDA standard plots. The concentration of LPO determined with and without treatment of FBHBE was showed in Figure 6. The Figure 6 indicates the gradual increase of concentration of LPO as dose dependent manner. Low and middle dose showed moderate increase whereas high dose 75 µg/mL showed elevated concentration of LPO compared to control.T

Gene expression studies

The effect of FBHBE on apoptotic related genes were studied. The gel image was depicted in Figure 7. Apoptotic pathway related genes, such as Bax, PARP and c-MYC genes were chosen for the analysis. The Figure 7 showed, the negligible level of gene expression was observed in control group for the pro apoptotic gene Bax, whereas the expression level was increased significantly as dose dependent manner for the treated groups. In 75 µg/mL, the predominant expression was observed in Bax where as in PARP and c-MYC gene, there was down regulation of expression was observed as dose dependent manner. Beta actin gene was kept as reference. The upregulation of Bax and down regulation of PARP and c-MYC provides the further confirmation of anti cancer property of FBHBE which is inlined with the aforementioned results.

DISCUSSION

Cancer, the predominant causative of death globally and its metastasis property leads the severity condition in cancer patients. People all over the world was suffered by many types of cancers, where the lung cancers stand the second most cause of death reported by WHO.[1] Anti-cancer drugs are more acquirable for sale in the market which can cause the dreadful effects in cancer patients. Another significant challenge in commercial drugs is to target cancer cells while leaving normal cells undisturbed. Scientists and pharmaceutical companies were investigating targeted drug delivery in the cancer condition. It is challenging to create a chemically derived medication, nevertheless, that is selective to cancer cell cytotoxicity and non-toxic to normal cells. As a result, there is a significant demand for the creation and research of naturally derived substances for the treatment of cancer, with a focus on those derived from plant species and their natural products.[21,22] To overcome these situations, a focus on folk medicine is required. Most of the herbal plants possess anti-malignant and antioxidant properties, which can inhibit the proliferation and metastasis of cancer cells. In this work, we

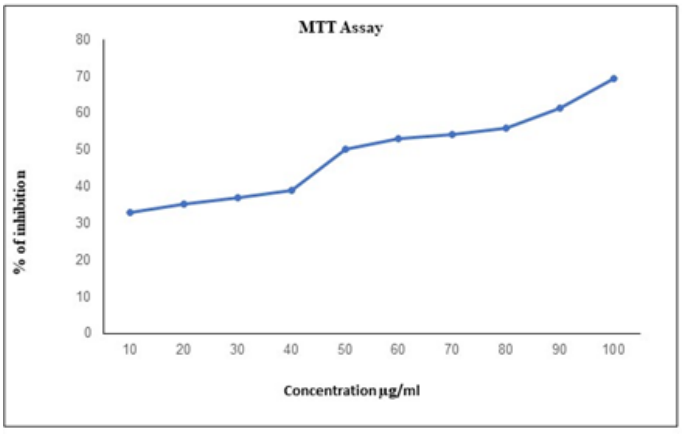

Figure 1: Cytotoxicity analysis. The figure denotes the graphical representation of cytotoxic effect of FBHBE against A549 cell line.

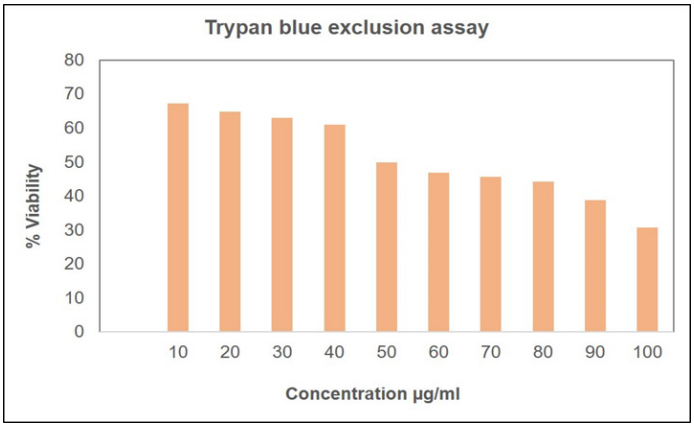

Figure 2: Cell viability-Trypan Blue method. The figure shows the cell count of A549 cell line with various concentration of extract. The decreasing cell count observed with increase in dosage concentration.

investigated the anti-proliferation property of *Ficus benghalensis* hydroalcoholic bark extract against the lung cancer cell line. Many studies have been conducted earlier with other parts of the *F. beghalensis* plant in various cell lines. The cytotoxicity evaluation of *F. beghalensis* bark was studied at very beginning level in lung cancer cell lines A549 and the cytotoxic potency of *F. beghalensis* bark was poorly understood. So, the aim of this investigation is to evaluate the anti-malignant property of *Ficus benghalensis* hydroalcoholic bark extract (FBHBE) against A549 cell line as primary screening by MTT cytotoxicity assay followed by in-depth analysis through nuclear staining, oxidative stress markers level and pro apoptotic gene response studies.

Extraction of phenolic compounds needs different solvents, (i.e.) Ethanol: water (70:30) combinations could extract tannins, polyphenols, polyacetylenes, flavonoids, terpenoids, sterols, and alkaloids from plants and herbs effectively.[3,23] So, in this study, *Ficus benghalesis* bark was subjected to hydroalcoholic extraction method to extract its maximum phytochemical profile.

Antioxidant potency of FBHBE was analyzed using DPPH and Hydrogen peroxide assay which showed, it has marked level of activity to scavenge the free electrons compared with standard-Vitamin C. Antioxidant properties play a key role in the prevention of cancer like chronic diseases.[24] The phytochemicals present in the extract such as polyphenols and flavonoids play important role in donating electrons to the unpaired electrons to stabilize the homeostatic condition. Some of the free radicals are H+, OH-, NO-, Cl-, O2-, ROO- etc.[25] FBHBE possess high anti-oxidant activity which can help to balance the oxidative stress environment.

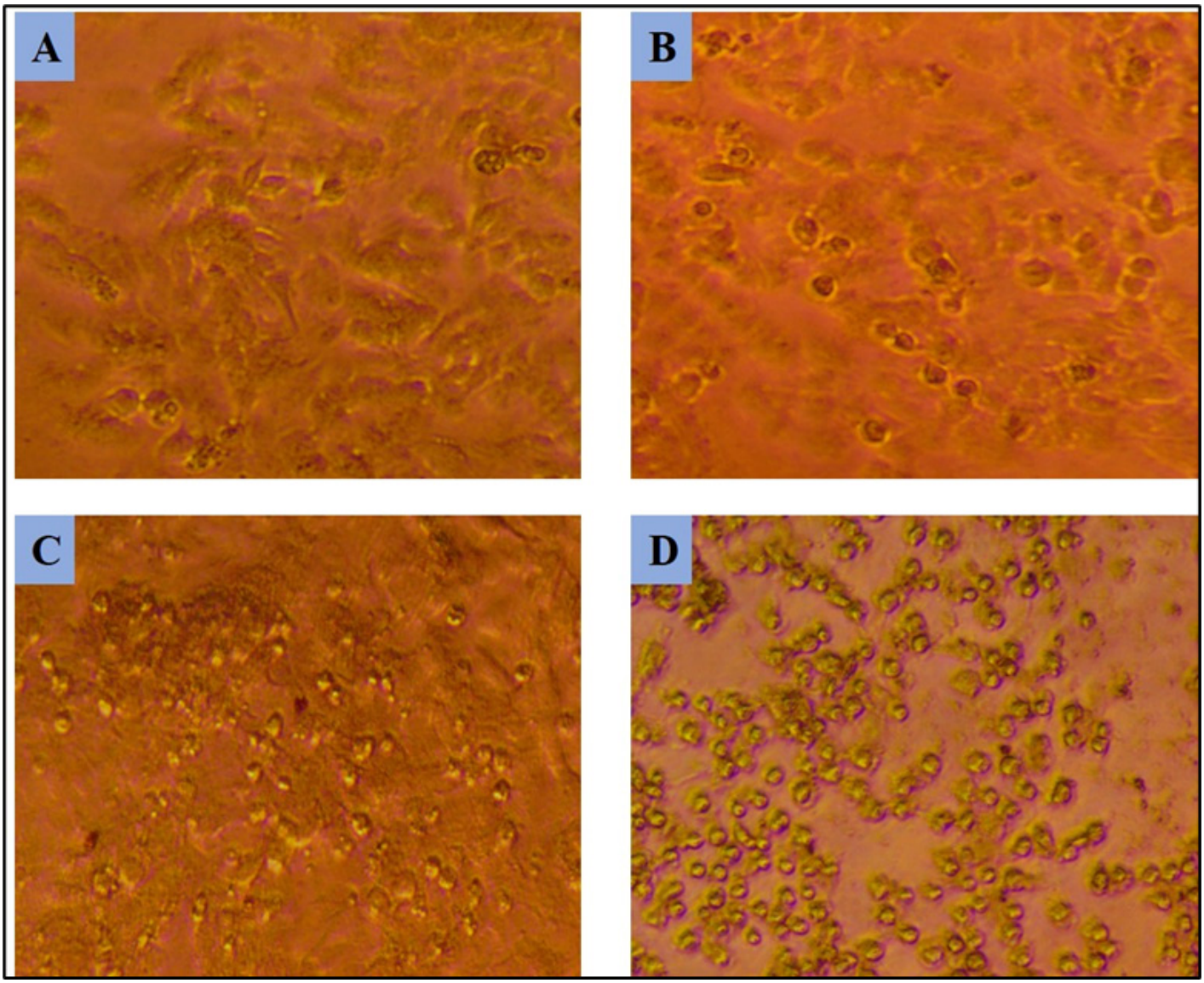

Figure 3: Morphometric analysis. Figure showed the phase contrast images of A549 cell line upon treated with FBHBE. A-Control, B-25 µg/mL, C-50 µg/mL and D-75 µg/mL. In control, cells exhibited the normal morphological structure whereas in treated, the morphological disintegration was noticed in the dose dependent manner.

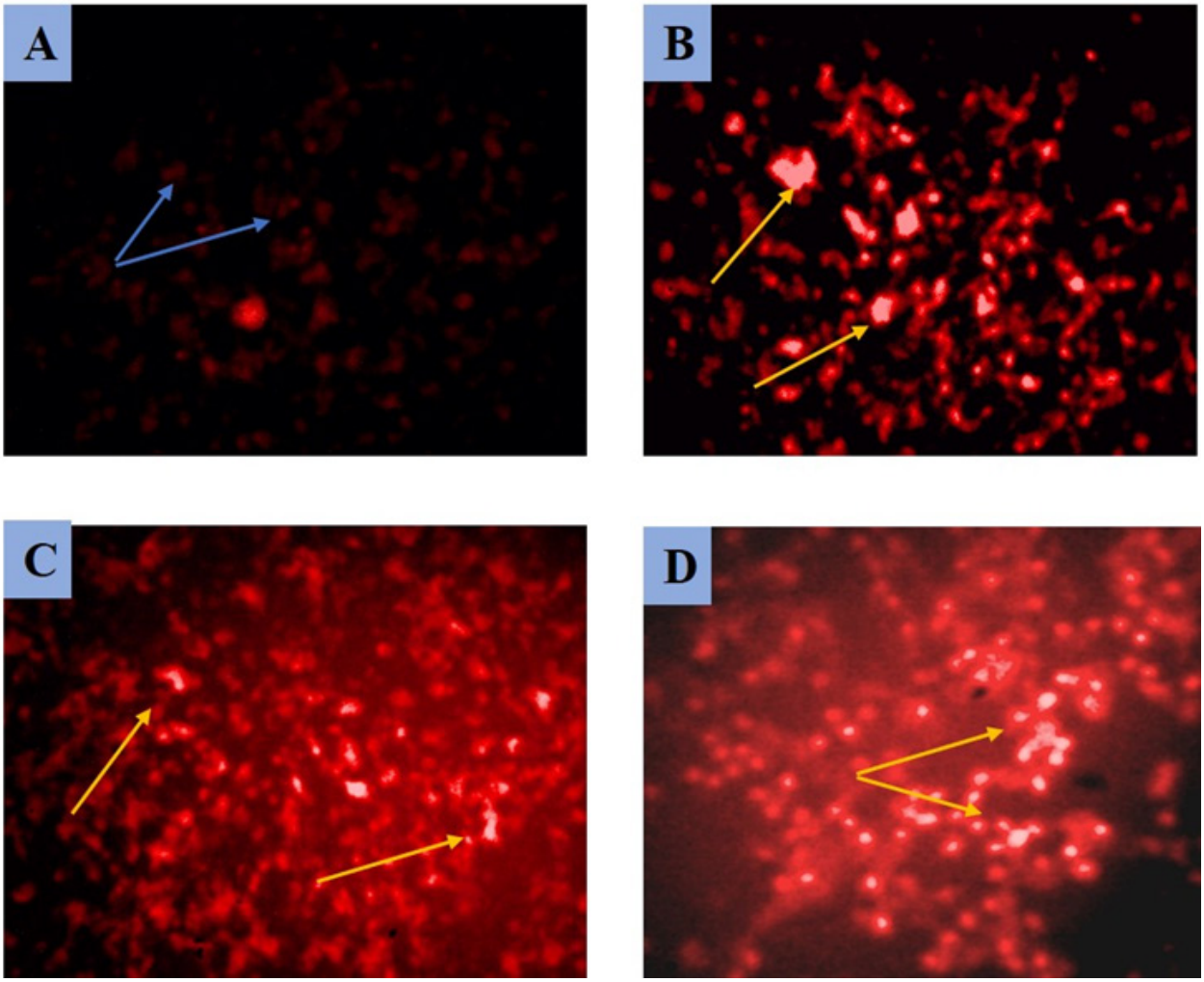

Figure 4: PI staining Images. The figure showed the nuclei staining images of treated and untreated A549 cell line. A-Control, B-25 µg/mL, C-50 µg/mL and D-75 µg/mL. The control image showed the fine nuclei morphology. Significant increase in the number of condensed nuclei and apoptotic bodies which showed as dark red in color was observed in treated groups. The colour intensity was increased with dose respectively which indicates the increasing rate of apoptosis. Blue arrow indicates the normal cell nuclei and yellow arrow indicates the condensed nuclei.

Primary screening of cytotoxicity property was evaluated by MTT assay, which showed IC_{50} value-50.127% of inhibition at 50 µg/mL of extract. Khanal and Patil, 2020 research work states that the Chinese herb of *Ficus benghalensis* (FBHBE) showed IC_{50} value at 193.78±6.58 µg/mL in A549 cell line.[25] Furthermore, the cell viability assay corroborates with the previous results. Trypan blue assay showed 49.88% of cell viability at 50 µg/mL.

The nuclear condensation, chromatin condensation, cell shrinkage, cell blebbing and apoptotic bodies formations were observed in morphological analysis and also through Propidium Iodide staining assay. The apoptotic cell count was increased significantly based on the extract concentration. The red stain (PI) strongly binds with the DNA, that can enter only in dead cells via damaged plasma membrane and unable to enter into live cells.[5] The extract causes the damage in structural integrity of cell membrane by downregulating the cyto-skeletal mediated genes. The loss of cell membrane integrity makes dramatical decrease in intracellular ATP leads to the osmotic imbalance in cell membrane. This in turn leads to apoptosis by strong oxidative stress.[32]

The oxidative stress level of lung cancer cell line after treatment with FBHBE was studied with the help of estimation of nitric oxide and lipid peroxidation. The results of these assay indicated the significant increase in the production of NO and LPO in dose dependent manner. The polyphenols present in FBHBE had the tendency of elevating the oxidative stress in the cancer cells which leads to the formation of enormous free radicals called ROS. OH-, O_2- and H_2O_2 are the most predominant free radicals that increased the imbalance in homeostatic environment and modifications in cell signaling and metabolism. Consequently, ROS induces the inactivation of lipid metabolism, inactivation of intracellular proteins, mitochondrial dysfunctions

Table 1: Primer Sequences.

Sl. No.	Primer	Forward Sequence (5′-3′)	Reverse Sequence (3′-5′)
1	Bax	CGTGTCTGATCAATCCCCGA	GAGGCCAGAAGGCAGGATTG
2	cmyc	TCTCCGTCCTCGGATTCTCT	TGAGCTCCCAAATCTCTCCAG
3	PARP	CCCAGCCTTGTGGAAAACAC	CACCTGCAGAGACAGGCATT
4	β-actin	TCAAGGTGGGTGTCTTTCCTG	ATTTGCGGTGGACGATGGAG

Table 2: Radical scavenging activity of FBHBE.

Protocol	Standard & Sample	IC_{50} conc (µg/mL)	Replicate I	Replicate II	Mean±SD
DPPH Assay	Ascorbic Acid	40	51.24	50.11	50.67±0.79
	FBHBE	40	49.08	49.77	49.42±0.48
H_2O_2 Assay	Ascorbic Acid	60	47.03	46.87	46.95±0.11
	FBHBE	60	48.72	48.61	48.66±0.07

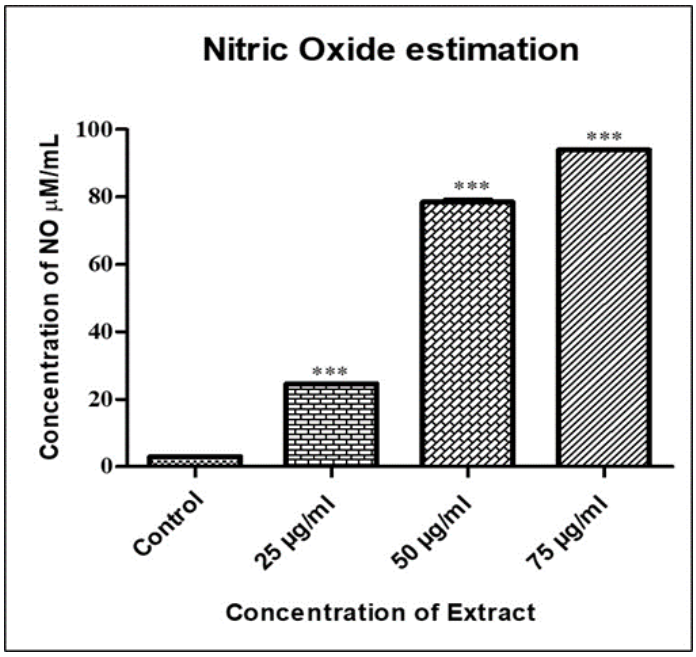

Figure 5: Nitric oxide estimation. Figure showed the NO release in A549 cell line with and without treatment of FBHBE. The figure indicated that there was significant increase in the release of NO as dose dependent manner.

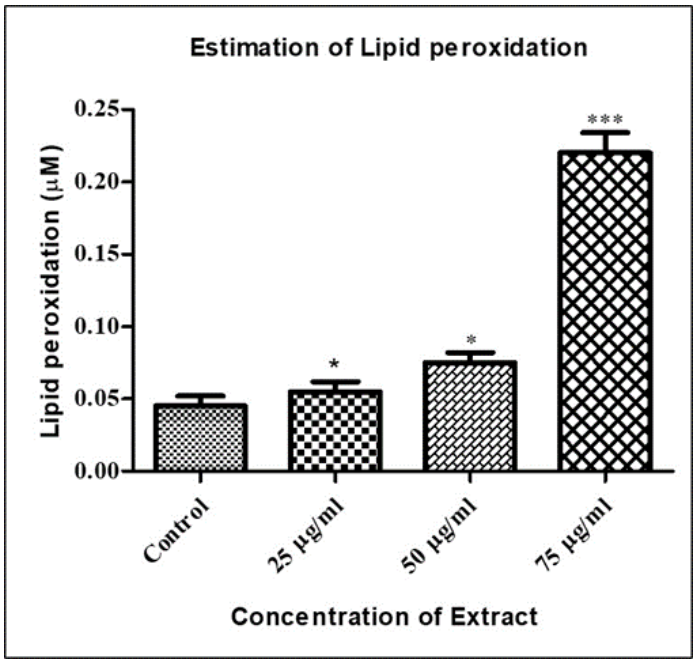

Figure 6: Lipid peroxidation estimation. Figure showed LPO estimation in A549 cell line with or without treatment of FBHBE. LPO release increased moderately in low and middle dosage range (25 and 50 µg/mL). In 75 µg/mL LPO increase was very high compared to control.

and DNA damage etc. which will lead to the apoptosis or necrosis.[26] Furthermore, lipid peroxidation products such as lipid hydroperoxides and aldehydes interacts with receptors of the membranes and transcription repressors in order to trigger both intrinsic/mitochondrial and extrinsic apoptotic signaling pathway, that can cause apoptosis by mediating cell death and decreasing proliferation.[27] The FBHBE treated lung cancer cell line showed the lack of balance between production and detoxification of ROS. This would be the main reason for the elevation of NO and LPO level. So, the phytoconstituents such as phenolic, flavonoids, aldehydes, keto groups, etc., present in the extract leads to the terrible homeostatic imbalance in lung cancer cell line which downregulates the signaling mechanisms leads to apoptosis.

The molecular level investigation also performed for the further evidence of anti-cancer property of FBHBE. The pro apoptotic gene Bax showed the upregulation that indicates that the activation of apoptotic pathway via cytochrome C release. Bax protein belongs to Bcl II family, which creates imbalance in mitochondrial membrane potential that leads cytochrome C release in cytoplasm that activates the cascade pathway leads to

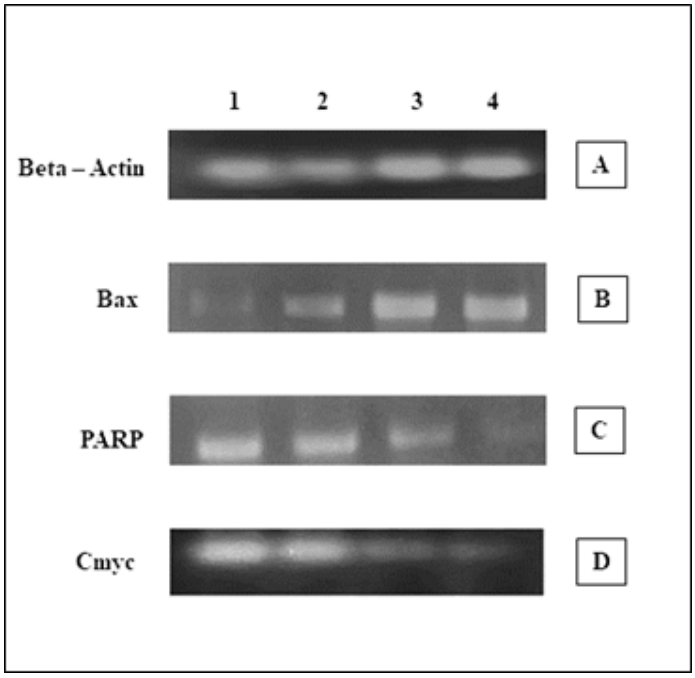

Figure 7: Gene expression analysis of A549 cell line with FBHBE. Gel image showed the gene expression levels of A549 with or without treatment of FBHBE. The expression level was increased significantly depends on the dosage in Bax and the reversible response was obtained in case of PARP and Cmyc respectively. Figure A showed Beta actin gene for reference. Lane 1-control, Lane 2-25 µg/mL, lane 3-50 µg/mL and Lane 4-75 µg/mL.

the apoptosis of cancer cells.[28] The upregulation of pro apoptotic genes indicated that the elevation of ROS indirectly suppresses the proliferative signaling mechanism which leads to the cell cycle arrest in S and M phase and disturbance in G0/G1 phase.[29]

PARP and c-MYC genes were analyzed and the down regulated expression was observed in dose dependent manner. PARP1 is the protein that involved mainly in DNA repairing process which is induced by NO mediated signal. The down regulation of PARP indicated the damaged DNA from one side and the increasing level of NO in other side leads to the inhibition of DNA damage repair leads the cancer cells to death.[30] c-MYC is the multi-functional transcription factor involving in wide range of cellular mechanism. The myc gene mainly involved in cell proliferation mechanism and the over expression of myc gene leads to the transformation of cell metabolisms.[31,32] So, the myc gene downregulation indicates the anti-proliferative property of FBHBE. Hence, the gene studies revealed that the anti-cancer mechanism of FBHBE extract in lung cancer cell line.

CONCLUSION

The study enlightens the anti-proliferative and anti-cancer property of *Ficus benghalensis* hydroalcoholic bark extract against lung cancer cell line A549. The cytotoxic effect of FBHBE was confirmed by viability and MTT assay. Morphometric and fluorescence staining revealed the deformation of cells upon treatment with FBHBE. The oxidative markers estimation further proved the increased stress level exhibited in FBHBE treated groups and the molecular level analysis evidenced the cytotoxic potency of FBHBE by upregulation of pro apoptotic gene and downregulation of DNA repair and proliferative gene. The anti-cancer property might be due to the rich profile of phytoconstituents of *Ficus benghalensis* bark as stated in previous literatures. Flavonoids and polyphenols may attribute the cytotoxic activity. Further studies need to be developed to study the remarkable active compounds behind this property of *Ficus benghalensis* bark and also elucidate the mechanism of action in cancer cell signaling pathways.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **EDTA:** Ethylenediamine tetra acetic acid; **PBS:** Phosphate buffered saline; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl sulfoxide; **DMEM:** Dulbecco's Modified Eagle Medium; **LPO:** Lipid peroxidation; **NO:** Nitric oxide.

SUMMARY

The current study intended to evaluate Anti-proliferative and Antioxidant Potency of *Ficus benghalensis* Hydroalcoholic Bark Extract (FBHBE) against Lung Cancer Cell Line – A549

- *Ficus benghalensis* bark extract inhibits the proliferation of A549 cells after being treated for 24 hrs.
- FBHBE possess high anti-oxidant activity.
- On treating A549 cells with FBHBE, oxidative stress markers such as Lipid peroxidation and Nitric oxide were significantly elevated.
- The Pro-apoptotic gene Bax showed upregulation while Apoptotic genes like PARP and c-MYC were down regulated with increasing concentration.
- FBHBE phytoconstituents, flavonoids and polyphenols possess potential anti-cancer activity.

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