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ANTICANCER EFFECT OF *Coleus amboinicus* (KARPOORAVALLI) ON HUMAN LUNG CANCER CELL LINE (A549)

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ABSTRACT

Cancer is a major public health problem in both developed and developing countries. Plant derived products are being used for the treatment of cancer. Aromatic plants of lamiaceae family are known for its traditional medicinal value. The leaves of the plants are promoted in relieving cold, coughs, laryngitis, bronchitis, nasal congestion, inflammation of the mouth and throat. The leaves of *Coleus amboinicus (C.amboinicus)* have antioxidant, antibacterial, antimutagenic and anticancer activity. Ethanolic extract of *C. amboinicus* leaves were studied for the *invitro* cytotoxicity against human lung cancer cell line (A549) along with normal human lung cell line (VERO). The results showed that *C. amboinicus* treated A549 cell line with maximum cytotoxicity, Normal cell line (VERO) showed moderate cytotoxicity. Investigation demonstrated that *C. amboinicus* significantly suppressed growth and induced apoptosis in human lung cancer (A549) cell line.

INTRODUCTION

Cancer is still a growing health problem world-wide characterized by the irregular proliferation of the cells, as a cell progresses from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in cells behavior (Ashworth *et al.,* 2011). However, cancer to some degree is a preventable disease, as cancer risk can be reduced by avoidance of cancer causing biological, chemical, and physical agents, in addition to the habitual consumption of cancer protective foods (Amin *et al.,* 2008). Medicinal plants occupy an important position for being the paramount sources of drug discovery in the modern era. Plants have been indispensable in treating diverse forms of diseases including cancer. According to World Health Organization (WHO) 80% of the people living in the rural areas depend on medicinal plants as primary health care system (Farnsworth *et al.,* 1985). These practices are closely based on the knowledge of traditional use of medicinal plants. Natural herbal products are formulated with a combination of constituents from plants. Different types of effective drugs are being used to enhance anticancer activities. Proper understanding of the complex synergistic interaction of various constituents of anticancer herbs, would help in formulating the design to attack the cancerous cells without harming the normal cells of the body (Saxe, 1987). There are numerable scientific studies that have focused on the pharmacological activity of bio-active components from plants, increasing interest from scientific community as cancer suppressant. Epidemiological studies suggest that the daily intake of certain phytochemicals can reduce the incidence of several types of cancers (Russo *et al.,* 2010). Thus, chemoprevention by dietary phytochemicals emerges as one of the most promising approaches for reducing risk of cancer development. On the other hand, phytochemicals act in synergy with chemotherapeutic drugs to overcome cancer cell drug resistance, and further application of specific phytochemicals may also allow the use of lower

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concentrations of drugs in cancer treatment with an increased efficacy (Liu, 2004). *Coleus amboinicus* is used in traditional medicines (Ayurveda) native to South Indian Homeopathy, Unani and Siddha. It is used for the treatment of cough, cold and fever (Rout *et al.*, 2010), kidney and bladder stones, bone fractures, asthma, bronchitis, indigestion, diarrhoea, dysentry, insect bite, fever and cholera (Kumar *et al.*, 2008). Bataknese lactating women traditionally consume leaves of *Coleus amboinicus* in the first month after child birth to stimulate breast milk production (Damanik *et al.*, 2006). The Malay community use the juice or decoction of the leaves for pain relief around stomach and heart. The Chinese community of Peninsula use the juice with sugar for cough treatment (Garcia *et al.*, 1973; Lukhoba *et al.*, 2006). It is applied externally for burns and insect bites, internally as a carminative and also to control asthma by South Indian community (Kiruba *et al.*, 2006; Pritima *et al.*, 2008). The aromatic leaves are used for flavoring meat, soups and fish and are eaten as a vegetable in most tropical countries and Africa. Pushpa kumari and Ranganayakulu (2008) reported anti- epileptic and antioxidant activity of *Coleus amboinicus* leaf juice. *In vivo* and *in vitro* study showed that the essential oil of *C.amboinicus* has a significant antioxidant activity due to the presence of important bioactive compounds and can be subjected for pharmaceutical drug formulations. This study would unfold the antioxidant activity and anticancer effect of *Coleus amboinicus* leaf extract in order to ascertain the scientific proof for its traditional use.

MATERIALS AND METHODS

Obtaining of cell line and its culture

Normal- VERO and Lung cancer (A549) cell lines were obtained from Department of Animal Biotechnology, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamilnadu. The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 U/ml), and Streptomycin (100 U/ml) in a humidified atmosphere of 50 µg/ml $C0_2$ at 37°C.

Reagents

Chemicals were purchased from various laboratories. MEM (Hi Media), FBS (Cistron), Trypsin, methylthiazol diphenyl - tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) (Sisco, Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

In vitro assay for cytotoxicity activity (MTT assay)

The cytotoxicity of Normal VERO and lung cancer cell line A549 were determined by the MTT assay (Mosmann, 1983). Cells (1 x $l0^5$/well) were plated in 1.0 ml of medium /well in 24-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 48hrs at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200ul/well (5mg/ml) of 0.5% 3- (4, 5-dimethyl-2-thiazolyl) -2, 5-disphenyl—tetrazolium bromide cells (MTT) solutions was added. After 4hr incubation, 0.01M HCL/isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurement were performed and the concentration required for a 50% inhibition of viability lethal concentration (IC50) was determined graphically. The absorbance at 570 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 and A549 were expressed as the % cell viability, using the following formula.

RESULTS

The *in-vitro* cytotoxicity effect of ethanol extract of *Coleus amboinius* leaves are given in Table (1-3), Graph (1-3) and Fig (1-3). The maximum cell viability 91.82% (minimum cell death 8.18%) was observed in 7.8 µg/ml of plant extract at 24 hrs of experiment in normal lung cell line (VERO). The minimum cell viability of 19.58% (maximum cell death 80.42%) was also observed in 1000µg/ml concentration of plant extract at 24 hrs. The IC_{50} was 125 µg/ml at 24 hrs (Table 1, Graph 1 and Fig 1).

Table 1. Cytotoxicity effect of Sample on VERO Cell line (24 Hrs)

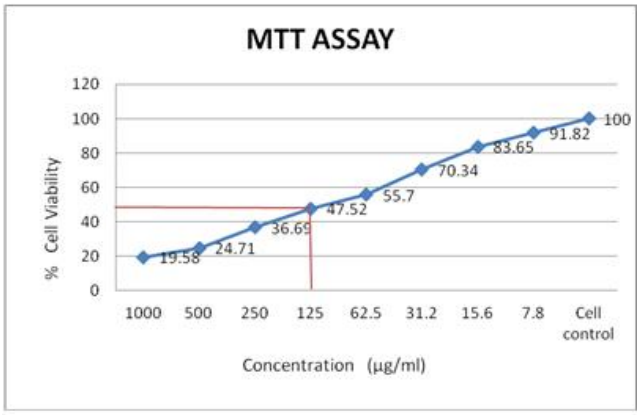
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.09	0.10	0.11	0.103	19.58
2	500	0.14	0.13	0.12	0.13	24.71
3	250	0.19	0.19	0.20	0.193	36.69
4	125	0.26	0.25	0.24	0.25	47.52
5	62.5	0.30	0.29	0.29	0.293	55.70
6	31.2	0.36	0.37	0.38	0.37	70.34
7	15.6	0.43	0.44	0.45	0.44	83.65
8	7.8	0.49	0.48	0.48	0.483	91.82
9	Cell control	0.52	0.53	0.53	0.526	100

Table 2. Cytotoxicity effect of sample on VERO cell line (48 Hrs)

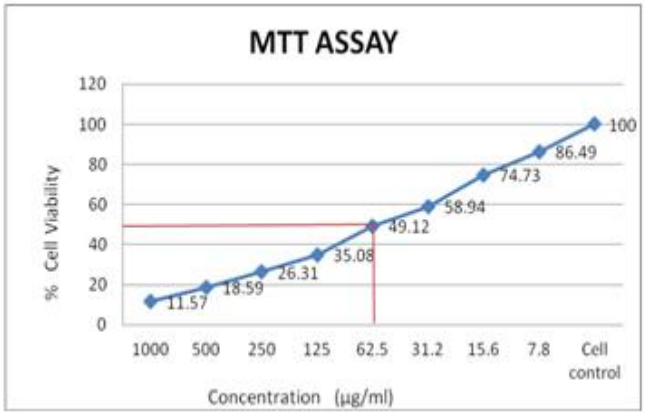
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.07	0.07	0.06	0.066	11.57
2	500	0.10	0.11	0.11	0.106	18.59
3	250	0.16	0.15	0.14	0.15	26.31
4	125	0.20	0.19	0.21	0.20	35.08
5	62.5	0.27	0.28	0.29	0.28	49.12
6	31.2	0.34	0.33	0.34	0.336	58.94
7	15.6	0.42	0.43	0.43	0.426	74.73
8	7.8	0.50	0.49	0.49	0.493	86.49
9	Cell control	0.57	0.58	0.56	0.57	100

Table 3. Cytotoxicity effect of sample on VERO cell line (72 Hrs)

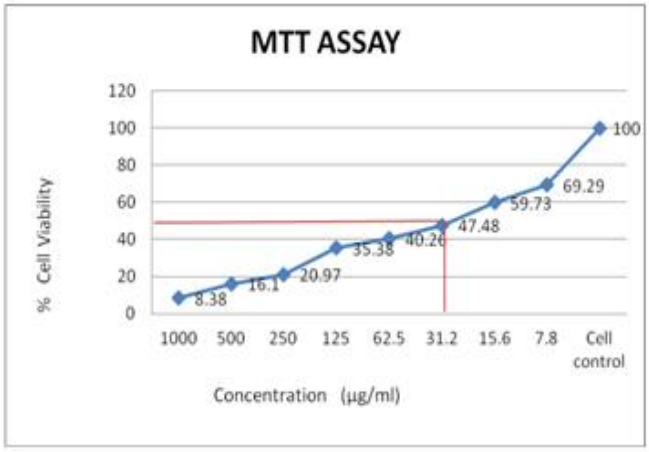
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.05	0.06	0.04	0.05	8.38
2	500	0.09	0.10	0.10	0.096	16.10
3	250	0.12	0.13	0.15	0.125	20.97
4	125	0.20	0.20	0.18	0.193	35.38
5	62.5	0.24	0.25	0.23	0.24	40.26
6	31.2	0.28	0.29	0.28	0.283	47.48
7	15.6	0.35	0.36	0.36	0.356	59.73
8	7.8	0.41	0.42	0.41	0.413	69.29
9	Cell control	0.59	0.60	0.60	0.596	100



Graph 1. Cytotoxicity effect of sample on VERO Cell line (24 Hrs)

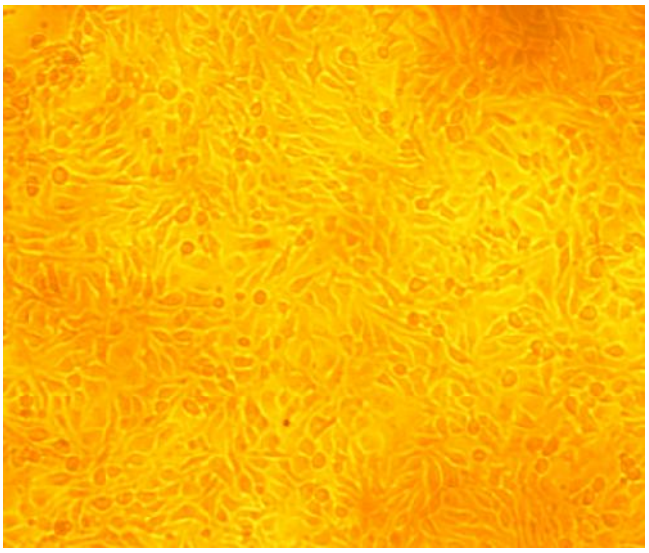


Graph 2. Cytotoxicity effect of sample on VERO cell line (48 Hrs)

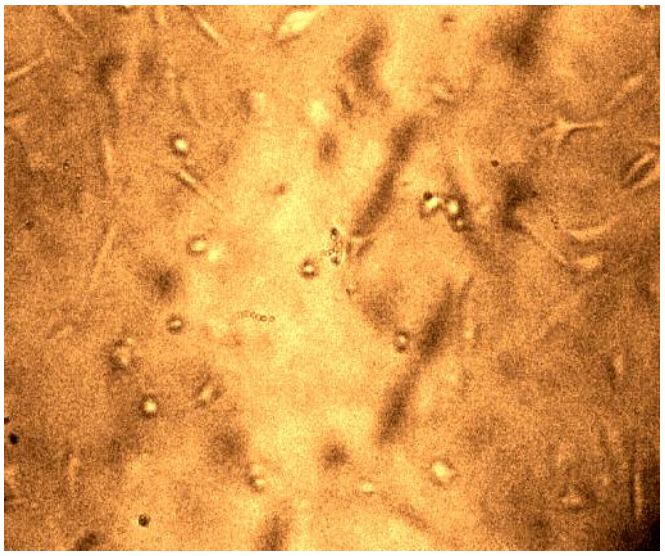


Graph 3. Cytotoxicity effect of sample on VERO cell line (72 Hrs)

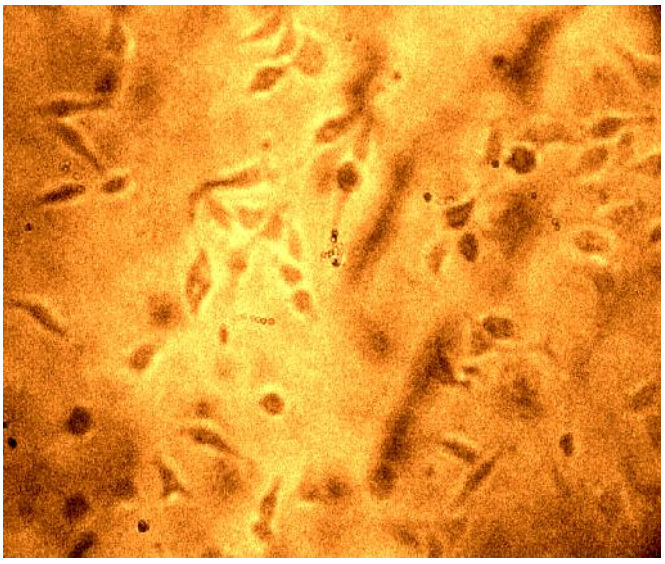
The *in-vitro* cytotoxicity of ethanol extract of *C. amboinicus* at 48 hrs the normal lung cell line (VERO) show maximum cell death 88.43% (minimum cell viability 11.57%) was recorded in 1000 µg/ml concentration of *C. amboinicus* leaf extract at 48 hrs of treatment. The minimum cell death 13.51% (maximum cell viability 86.49%) was observed in 7.8 µg/ml concentration of plant extract at 48 hours and the IC_{50} value 62.5 µg/ml at 48 hrs of experiment (Table 2, Graph 2 and Fig 2).



Normal VERO Cell line

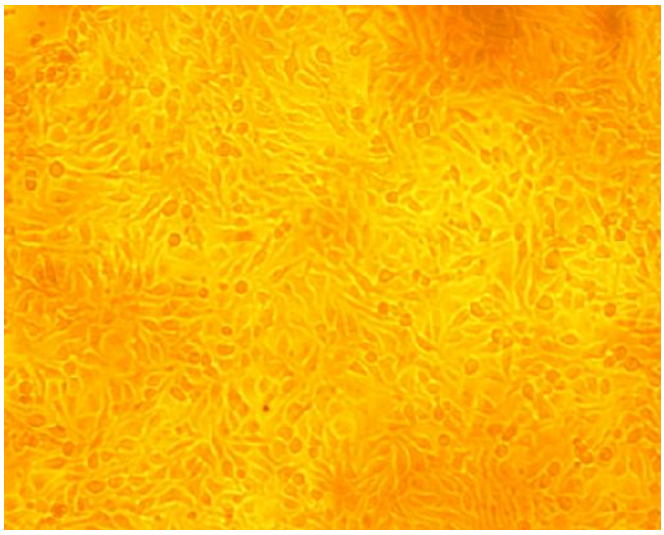


Toxicity- 125µg/ml

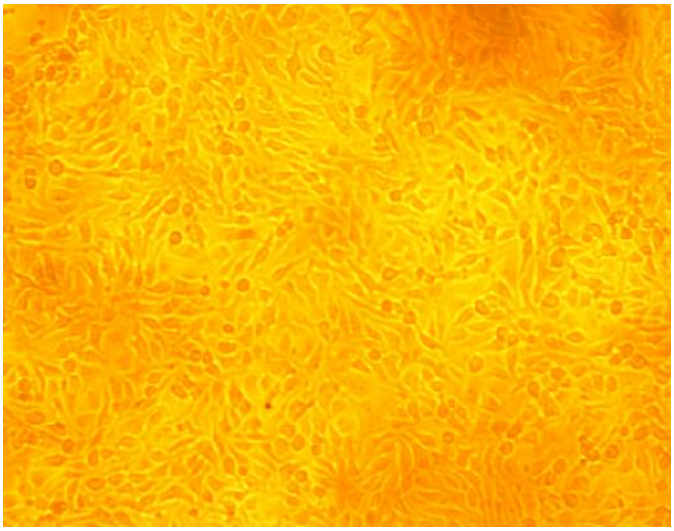


Toxicity- 31.2µg/ml

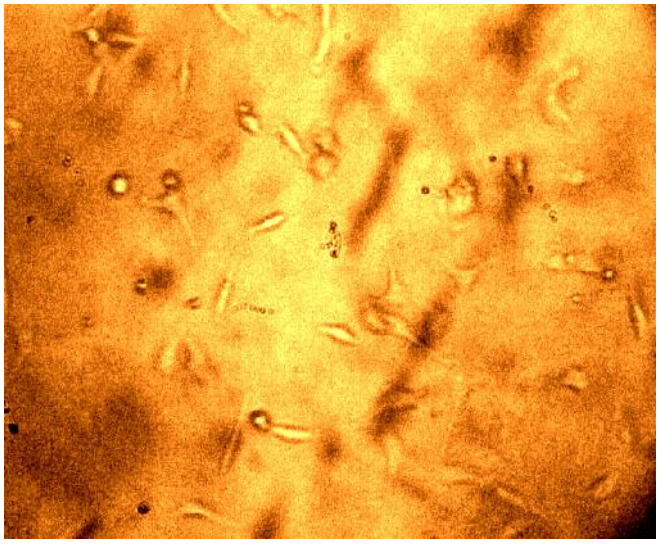
Figure 1. Cytotoxicity effect of Sample on VERO Cell line (24 Hrs)



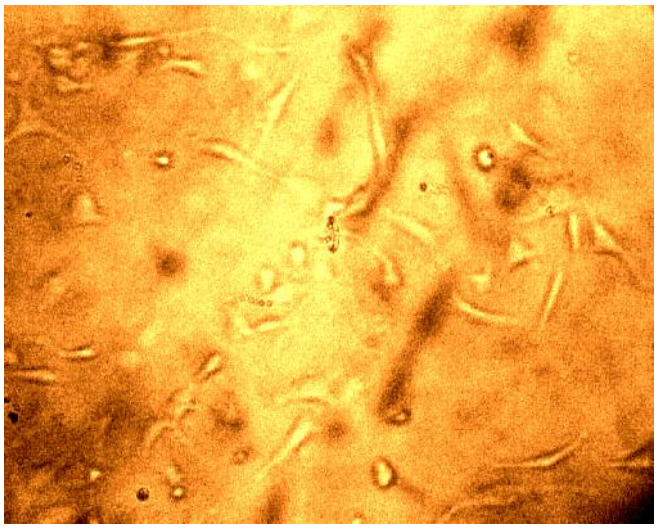
Normal VERO Cell line



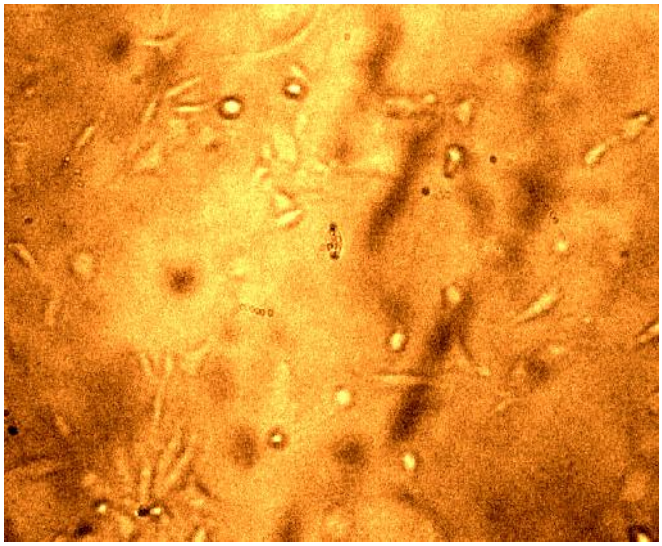
Toxicity- 31.2µg/ml



Toxicity-62.5µg/ml

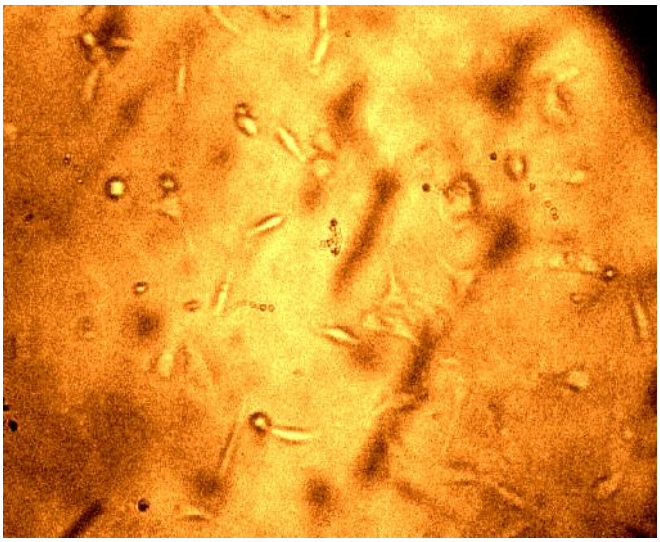


Toxicity- 31.2µg/ml



Toxicity- 31.2µg/ml

Figure 2. Cytotoxicity effect of Sample on VERO Cell line (48 Hrs)



Toxicity- 15.6µg/ml

Figure 3. Cytotoxicity effect of Sample on VERO Cell line (72 Hrs)

At 72 hrs of experiment the normal lung cell line (VERO) showed minimum cell viability 8.38% (maximum cell death 91.62%) was observed in 1000 µg/ml concentration of plant extract and maximum cell viability 69.29% (minimum cell death 30. 71%) was also observed in 7.8 µg/ml concentration of plant extract of 72 hrs of experiment. The IC_{50} value was 31.2 µg/ml at 72 hrs (Table 3, Graph 3 and Fig 3). In Human Lung cancer cell line (A549) the *in-vitro* cytotoxicity of ethanol extract of *Coleus amboinicus* are given in Table (4-6), Graph (4-6) and Fig (4-6). From the table the maximum cell viability 93.95% (minimum cell death 6.05%) was observed in 7.8 µg/ml concentration of leaf extract at 24 hrs. The minimum cell viability 14.11% (maximum cell death 85.89%) was observed in 1000 µg/ml concentration of plant extract at 24 hrs of experiment. The IC_{50} value was 62.5 µg/ml (Table 4, Graph 4 and Fig 4). The *invitro* cytotoxicity of ethanol extract of *Coleus amboinicus* at 48 hrs, the lung cancer cell line (A549) showed the minimum cell viability 7.96% (Maximum cell death 92.04%) was observed in 1000 µg/ml concentration of plant extract.

Table 4. Anticancer effect of sample on A549 cell line (24 Hrs)

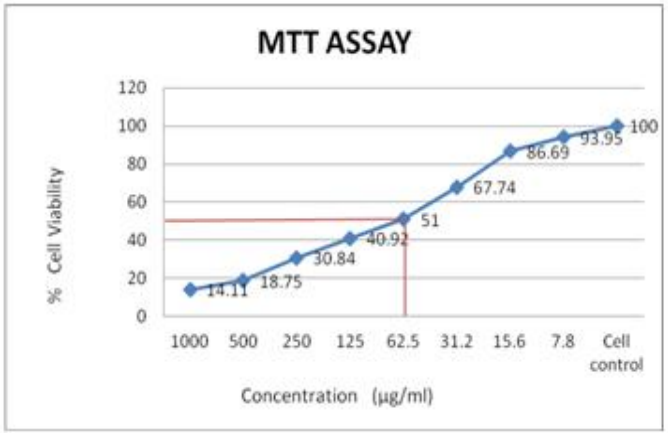
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.06	0.07	0.08	0.07	14.11
2	500	0.09	0.09	0.10	0.093	18.75
3	250	0.15	0.16	0.15	0.153	30.84
4	125	0.20	0.21	0.20	0.203	40.92
5	62.5	0.26	0.25	0.25	0.253	51.0
6	31.2	0.33	0.34	0.34	0.336	67.74
7	15.6	0.43	0.44	0.42	0.43	86.69
8	7.8	0.46	0.47	0.47	0.466	93.95
9	Cell control	0.49	0.50	0.50	0.496	100

Table 5. Anticancer effect of sample on A549 cell line (48 Hrs)

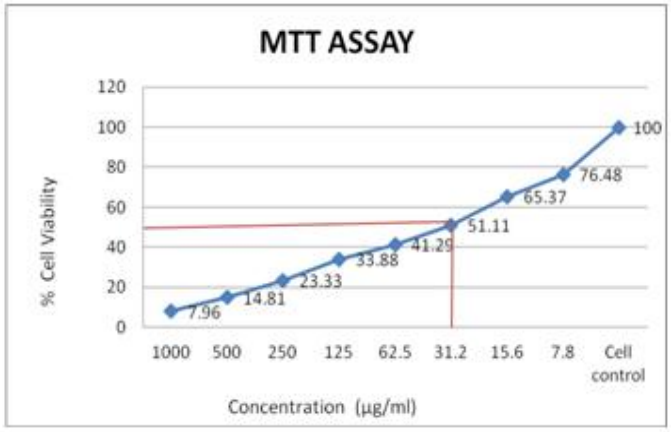
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.04	0.04	0.05	0.043	7.96
2	500	0.08	0.07	0.09	0.08	14.81
3	250	0.12	0.13	0.13	0.126	23.33
4	125	0.18	0.19	0.18	0.183	33.88
5	62.5	0.22	0.23	0.22	0.223	41.29
6	31.2	0.28	0.28	0.27	0.276	51.11
7	15.6	0.34	0.36	0.36	0.353	65.37
8	7.8	0.42	0.41	0.41	0.413	76.48
9	Cell control	0.54	0.54	0.54	0.54	100

Table 6. Anticancer effect of sample on A549 cell line (72 Hrs)

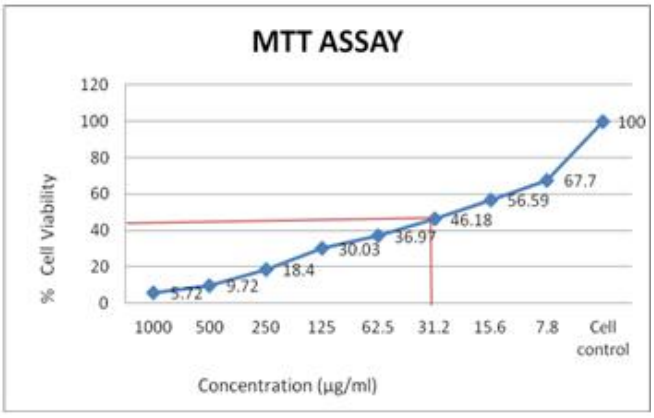
S.No	Concentration (µg/ml)	Absorbance(O.D)			Average	Cell viability (%)
1	1000	0.04	0.03	0.03	0.033	5.72
2	500	0.06	0.05	0.06	0.056	9.72
3	250	0.10	0.11	0.11	0.106	18.40
4	125	0.17	0.17	0.18	0.173	30.03
5	62.5	0.21	0.22	0.21	0.213	36.97
6	31.2	0.27	0.27	0.26	0.266	46.18
7	15.6	0.33	0.32	0.33	0.326	56.59
8	7.8	0.40	0.39	0.38	0.39	67.70
9	Cell control	0.58	0.58	0.57	0.576	100



Graph 4. Anticancer effect of sample on A549 cell line (24 Hrs)

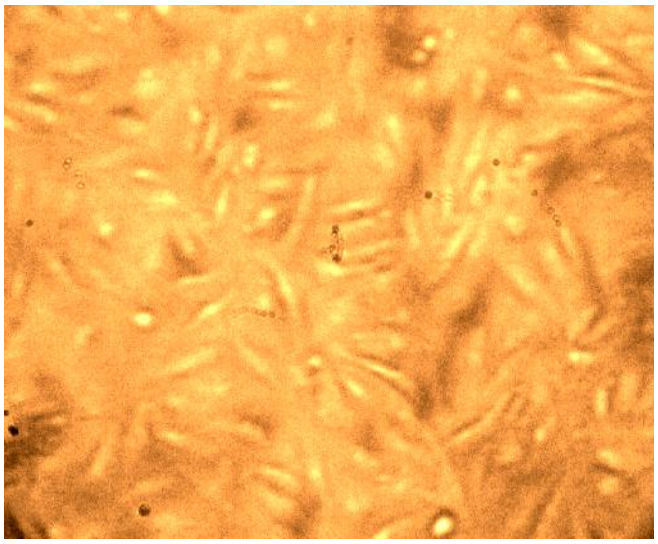


Graph 5. Anticancer effect of sample on A549 cell line (48 Hrs)

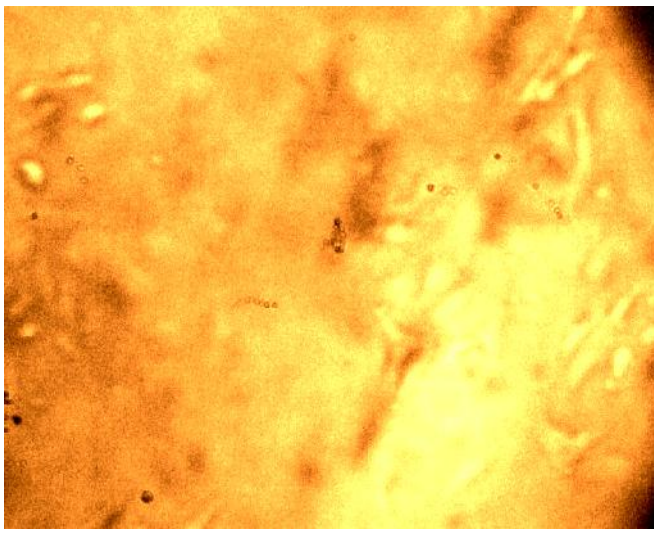


Graph 6. Anticancer effect of sample on A549 cell line (72 Hrs)

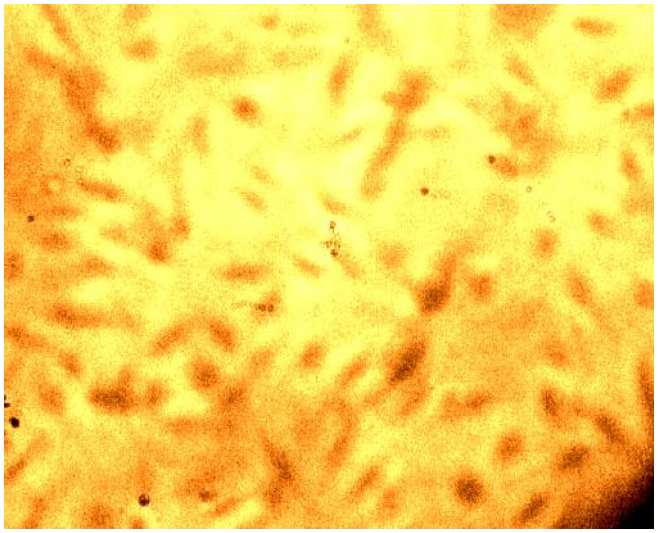
The maximum cell viability 76.48% (minimum cell death 23.52%) was recorded in 7.8 µg/ml concentration of plant extract at 48 hrs of treatment. The IC_{50} value was 31.2 µg/ml at 48 hrs (Table 5, Graph 5 and Fig 5). At 72 hrs of experiment, human lung cancer cell line (A549) showed maximum cell death 94.28% (minimum cell viability 5.72%) was observed in 1000 µg/ml concentration of leaf extract of *C.amboinicus*, the minimum cell death 32.30% (maximum cell viability 67.70%) was recorded in 7.8 µg/ml concentration of plant extract at 72 hrs of experiment. The IC_{50} value was 31.2 µg/ml (Table 6, Graph 6 and Fig 6).



Normal A549 cell line

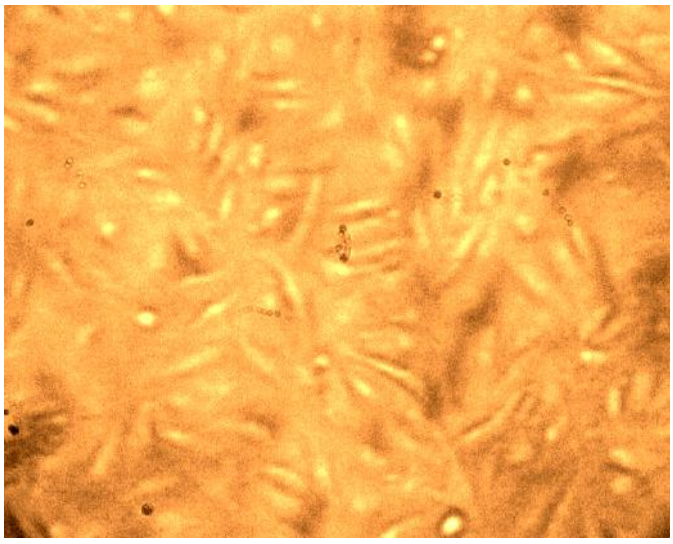


Toxicity- 1000µg/ml

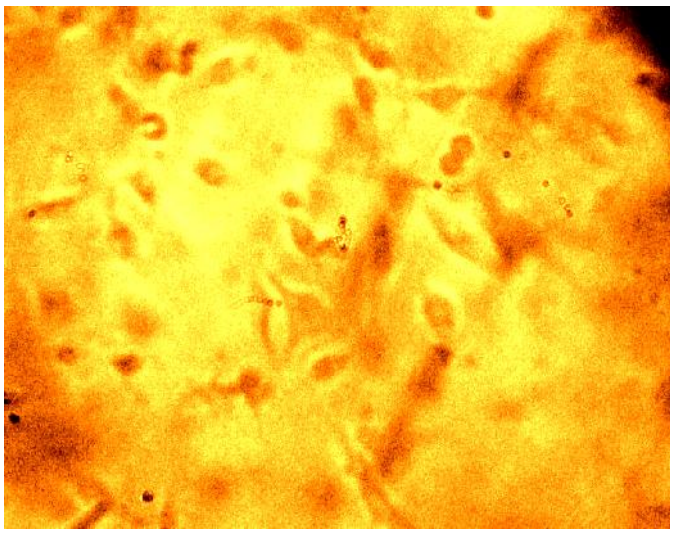


Toxicity- 62.5µg/ml

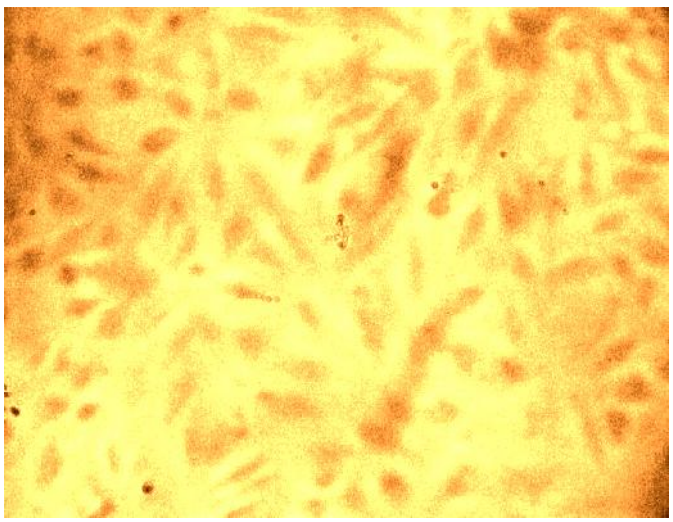
Figure 4. Anticancer effect of Sample on A549 Cell line (24 Hrs)



Normal A549 cell line

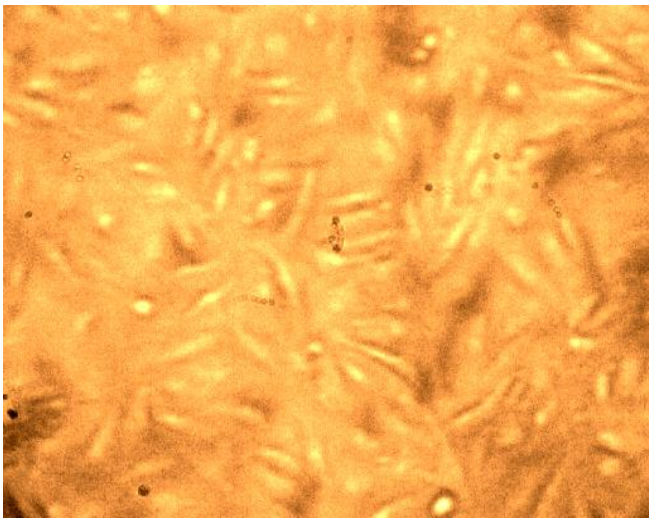


Toxicity- 125µg/ml

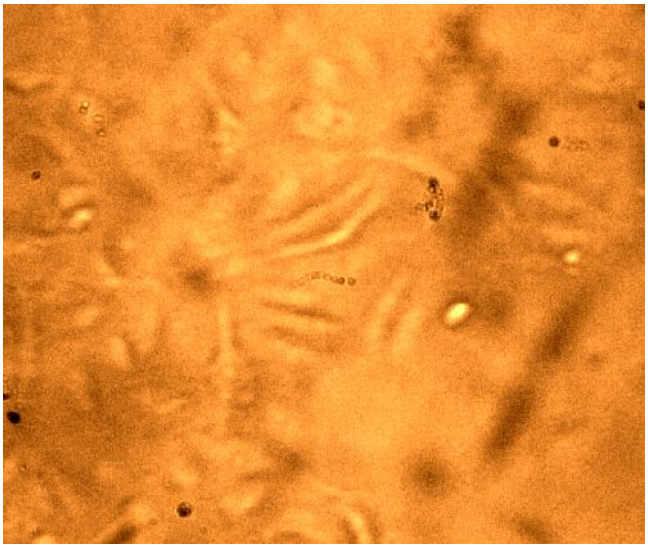


Toxicity- 15.6µg/ml

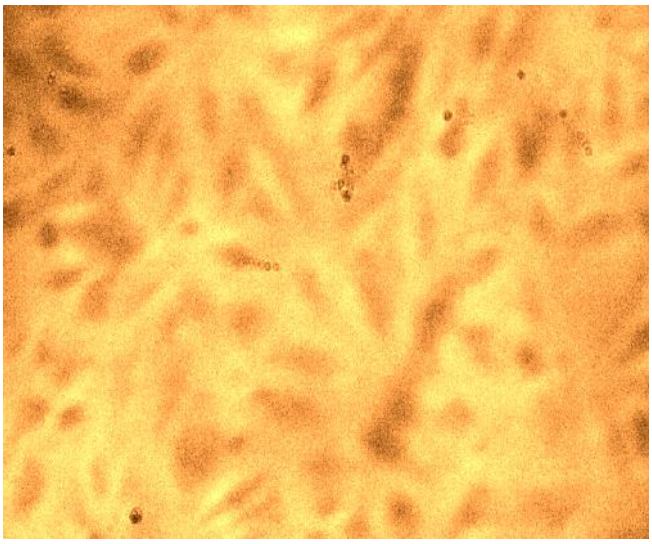
Figure 5. Anticancer effect of Sample on A549 Cell line (48 Hrs)



Normal A549 cell line



Toxicity- 125µg/ml



Toxicity- 15.6µg/ml

Figure 6. Anticancer effect of Sample on A549 Cell line (72 Hrs)

DISCUSSION

Cancer is believed to be the result of external fracture combined with heredity (Anderson *et al*., 1998). Non-lethal genetic damage by carcinogenic agents allows for the neoplastic transformations, such as self-sufficiency in growth signal, insensitivity to growth-inhibitory signal, evasion of apoptosis, limitless replication, sustained angiogenesis and the ability to invade and metastasize (Alberts *et al.*, 2002). Phytochemicals are increasingly used in treatment of cancer because of their availability and potential anti-cancer activity when compared with chemotherapy (Mohammad Mijanur Rahman and Asaduzzaman Khan, 2013). Biological targets of phytochemicals in mammalian cells were found to be involved in inflammatory processes and oncogenic transformation, such as the alteration of cell cycle control, apoptosis evasion, angiogenesis and metastasis (Surh, 2003). The plants belonging to lamiaceae family posses thymol, sesquiterpenes, flavanoids and aliphatic compounds. These compounds are known to act against cancer (Pushpa kumari and Ranganayakulu, 2008).

The anti cancer activity of ethanolic leaf extract of *Coleus amboinicus* was evaluated by *invitro* cytotoxicity method. The results showed 94.28% of human lung cancer cell line (A549) death in higher concentration of 1000 µg/ml of ethanolic *C.amboinicus* extract. The data of the results of the *in vitro* studies suggested the ethanol extract of lamiaceaeous plant *C. amboinicus* posses potent anticancer activity against human lung carcinoma. The results are in agreement with those of Gangai Abirami and Nirmala (2014). They studied anticancer effect of *Mentha piperita, Ocimum basilicum*, and *Coleus aromaticus* against Human laryngeal Epidermoid carcinoma (HEP-2) cell lines. They found the highest anticancerous property in *Mentha piperita* leaves due to the presence of the monoterpenes called D-carvone and D-limonene. D-carvone has been found to reduce stomach tumour formation and pulmonary adenoma formation induced by N-nitrosodimethylamine in mice (Zhiying *et al.*, 2008). Manjamalai and Berlin Grace (2012) investigated *in vivo* and *in vitro* antioxidant activities of *C.amboinicus* essential oil having important volatile constituents such as carvocrol, thymol, cis/trans – caryophyllene, and P-cymene they found out. Dayana and Parameswari (2014) investigated that the antoxidant and lipid peroxidation (LPO) potential of *Plectranthus amboinicus* leaf extracts by employing established *in vitro* systems- superoxide, nitric oxide, hydroxyl radical scavenging, total antioxidant and reducing power.

Investigation of the underlying pharmacokinetic mechanisms through which phyto-chemicals evoke their anti-cancer effect introduces a panel of molecular targets. This panel includes apoptotic proteins (caspases, bax etc), protein kinases (PKA, PKC, MAPK, TYK2 etc), anti-apoptotic proteins (bcl2, TRAF1, survivin etc), growth factors (TNF, EGF, FGF, PDGF etc), transcription factors (Apl, NF-κB, Nrf2, p53 etc), cell adhesion molecules (ICAM-1, VCAM etc) and cell cycle proteins (Cyclin D, CDK1, CDK2, p27, p21 etc). Moreover, phytochemicals interfere with multiple cell-signaling pathways (Agarwal *et al.*, 2006). They reported that the mechanism of caspase – 8 activation and mitochondrial mediated apoptotic pathways are involved in the action of d-limonene and d-carvone. Hydroalcoholic extract of *Plectranthus amboinicus* (HAPA), showed significant concentration dependent inhibition

potential of cell proliferation. The antioxidant ability suggests that the leaves of the plant could be effectively employed as an essential ingredient in treatment and curative nature of disease. HAPA showed significant inhibition of LPO and damage caused to the hydrophobic core of bio-membranes by oxidative stress. Extensive studies at molecular level are essential to decipher the *in vivo* safety of this extract in experimental animal models. The work of Manjamalai and Berlin Grace (2012) is of great interest. They found out the essential oil of plant *Plectranthus amboinicus* (Lour) possess a significant antioxidant property against stress created in cell line induced lung cancer model due to the presence of phytochemical compounds such as carvocrol and thymol. The present findings are supported by the work of Rashmi Sahay Khare *et al.* (2011), they concluded that *Coleus amboinicus* have rosmarinic acid, caffeic acid and chlorogenic acid are responsible for antioxidant activity.

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