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Original Article

# Apocynin Induces Apoptosis in Human Lung Cancer A549 Cells via Regulating Apoptotic Protein and Inflammatory Cytokines

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## ABSTRACT

**Objectives:** Lung cancer, widely considered the leading cause of cancer-related deaths world wide, ranks fourth in terms of its occurrence among all types of malignant tumors. Phytochemicals are the potent alternative for these chemotherapeutic drugs. They have proven to render anticancer effects against various cancers such as breast, liver, brain, lung, skin, bone etc., *in vivo, in vitro* and even in clinical phase trials. One such phytochemical is apocynin, polyphenolic compound obtained from the *Picrorhiza kurroa* roots. It is a persuasive anti-inflammatory drug utilized to treat inflammatory ailments like arthritis, inflammatory bowel disease, colitis, stroke etc., **Materials and Methods:** We demonstrated the anticancer activity of apocynin against lung cancer A549 cells. Cytotoxicity effect of apocynin at different dose against A549 cells were assessed with MTT assay. The ROS accumulation and impairment of mitochondrial membrane permeability by apocynin in A549 cells were analyzed with DCFH-DA and Rhodamine 123 staining techniques. Dual staining with AO/EtBr was done to detect the percentage of cell apoptosis induced by apocynin in A549 cells. DAPI staining was performed to detect the induction of nuclear fragmentation in A549. The apoptosis induction and inflammation in A549 cells by apocynin was studied by quantifying the apoptotic proteins and inflammatory cytokines using ELISA technique. **Results:** Apocynin significantly induced cytotoxicity against A549 cells in dose dependent. It enhanced the caspases, Bax levels and diminished the antiapoptotic protein Bcl2 level. Apocynin also decreased the proinflammatory cytokines thereby prevented the cancer cell growth and aided apoptosis induction in A549. The induction of apoptosis in A549 cells were confirmed with our staining techniques. **Conclusion:** Our findings of apocynin on A549 cells suggest apocynin may possess possible therapeutic efficacy in treating lung cancer patients.

**Keywords:** Lung cancer, Phytochemical, Apocynin, A549 cells, Apoptosis, Inflammation.

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## INTRODUCTION

Lung cancer is a common type of cancer that arises from the mucous membrane of the bronchi, glands, or alveolar epithelium.[1] Lung cancer, widely considered the leading cause of cancer-related fatalities globally, ranks fourth in terms of its occurrence among all types of malignant tumors. In 2020, lung cancer was the leading cause of mortality among all types of cancer.[2] There are two primary forms of lung cancer: small cell lung cancer, which is less prevalent and Non-Small Cell Lung Cancer (NSCLC), which is responsible for the majority of lung cancer occurrences.[3]

The incidence of lung cancer death worldwide is rapidly increasing due to the persistent adoption of cancer-causing behaviors such as smoking, physical inactivity and westernized diets. Lung cancer is the primary cause of cancer-related deaths in developing countries, surpassing other types of cancer such as colorectal, breast and prostate cancers.[4] Lung cancer cells exhibit fast growth and are susceptible to distant metastases. Conventional cancer treatments, including surgical techniques, radiation and chemotherapy, have been utilized to treat lung cancer. However, the current traditional methods have proven ineffective in prolonging the survival of cancer patients.[5] The chemotherapeutic drugs have serious impacts causing various allergic reactions, neuropathic syndromes and hand-foot syndrome. Prolonged usage of chemotherapeutic drugs also leads to drug resistance in lung cancer patients impairing the drug potency. Furthermore, there have been significant advancements in the treatment of lung cancer, the prognosis for

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patients remains poor, since the radiotherapy associated with less than 20% of 5-year survival rate.[6] Therefore discovering a drug with high potency and minimal side effect is the prime goal for pharmaceutical industries to treat lung cancer.

Polyphenolic compounds are the predominant class of phytochemicals with proven pharmaceutical properties. These polyphenolic compounds possess high specificity and minimal toxicity hence it is under research to treat various diseases. They are potent antioxidant and anti-inflammatory agents are tending to be safe to treat neurological diseases.[7,8] These compounds possess the property to alter the cancer cells epigenome thereby blocked cell cycle and triggered apoptosis in tumor cells. It also alters the antioxidant enzymes, apoptotic signaling protein in tumor cells inhibiting the proliferation growth and invasion of tumor cells.[9,10]

Apocynin is one such phenolic compound extracted from the *Apocynum cannabinum* and *Picrorhiza kurroa* roots which is a well-known inhibitor of NADPH-oxidase.[11] Numerous studies have proven the efficacy of apocynin on treating inflammatory disease such rheumatoid arthritis,[12] stroke,[13] atherosclerosis,[14] traumatic brain injury. It also shown promising effect on phase I clinical trial in treating anti-inflammatory diseases.[15] The anticancer efficacy of apocynin on lung cancer was not yet elucidated. In this work we assessed the potency of apocynin on human lung cancer cells.

## MATERIALS AND METHODS

### Chemicals and Reagents

Apocynin, with >98% of purity was procured from Sigma Aldrich USA. Supplements for culturing cells i.e., Dulbecco Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Dimethyl Sulfoxide (DMSO), TriZol reagent and antibiotic-antimycotic solution were obtained from ThermoFisher Scientific USA.

### Cell culture and Maintenance

Lung cancer cells (A549) were viewed under microscope to examine the confluency of cells and any other contamination. After confirming the cells are free of contamination, the medium was changed and incubated in the 5% $CO_2$ supplemented incubator at 37°C. The cells were cultivated with DMEM supplemented with 10% FBS and 1% antimycotic solution. The cells were trypsinized using Trypsin solution for further sub culturing.

### Cell cytotoxicity Assay

A549 cells were trypsinized with trypsin-EDTA and $1x10^4$ cells/mL were loaded on to 96-wellplate and incubated for 24 hr. The cultured cells were treated with diverse dosages of apocynin ranging from 10-100 µM/mL in triplicates and incubated for 24 hr at 37°C in the 5% $CO_2$ supplemented incubator. After 24 hr the cells were investigated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The 20 µL of MTT reagent with 5 mg/mL concentration was mixed to the cultured cells and incubated at 37°C in the 5% $CO_2$ supplemented incubator for 4 hr. After incubation period the culture medium was discarded and the formazan deposits was liquefied with 0.1 mL/well of DMSO solution. The final absorbance was taken at 490 nm. The experiment was repeated thrice and % of viability was determined by following equation

Viability (%)=OD of treated cells/ OD of control cellsX100

### Quantification of Reactive Oxygen Species (ROS)

ROS generated by the apocynin treated and control A549 cells were quantified using 2,7-Dichlorodihydrofluorescein Diacetate (DCFH-DA) staining technique (Invitrogen, USA). The A549 cells were exposed to 40 and 60 µM/mL dosages of apocynin for 24 hr at 37°C in 5% $CO_2$ supplemented incubator. After incubation the cells were treated with 10 mM DCFH-DA dye for 30 min. The stained cells were rinsed with Phosphate Buffered Saline (PBS) and monitored using fluorescent microscope. The images were then analyzed with ImageJ software and the proportion of fluorescence intensity was determined.

### Quantification of Mitochondrial Membrane Potential (MMP)

The MMP of control and apocynin treated A549 cells was investigated using Rhodamine-123 stain (Invitrogen, USA). The control, 40 and 60 µM/mL apocynin treated A549 cells were Rhodamine-123 dye for 30 min. Later, the cells were rinsed twice with PBS and observed using fluorescent microscope. The images were further assessed with ImageJ software to quantify the intensity of fluorescence.

### Quantification of Live/apoptotic cells

The apoptosis induction by apocynin in human lung cancer A549 cells was measured using dual staining technique. A549 cells were exposed with 40 and 60 µM/mL of apocynin for 24 hr at 37°C. The cells were then treated with 1 µg/mL of AO and EtBr (Sigma Aldrich, USA) in 1:1 ratio and incubated for 30 min. The cell was then washed with PBS and scrutinized using fluorescence microscope. The percentage of live and dead cells was measured using ImageJ software.

### Quantification of nuclear damage

The nuclear damage induced by apocynin on A549 cells were quantified using the nuclear stain DAPI (Invitrogen, USA). The 40 and 60 µM/mL of apocynin treated cell were stained with nuclear counterstain 300 nM DAPI and incubated for 15 min in dark. The stain was removed after incubation and rinsed with PBS thrice. The cells were then investigated using fluorescence microscope and percentage of apoptotic cells were quantified using ImageJ software.

### Quantification of apoptotic protein

The Caspase-3, Caspase-9, Bax and Bcl2 expressions were quantified using ELISA kits procured from Abcam, USA. Human lung cancer A549 cells were exposed to 40 and 60 µM dosages of apocynin for 24 hr at 37°C in 5% $CO_2$ supplemented incubator. Later, the cells were trypsinized and subjected to sonication. The sonicated cells were centrifuged at 10000 rpm for 10 min at 4°C. Supernatant was collected for further quantification of apoptotic proteins. The reagents and the standards were prepared freshly 30 min before the experiment. The ELISA plates were washed twice with the saline before the initiation of experiment. The 100 µL of sample was mixed to the wells in triplicates and incubated at 37°C for 45 min. After incubation the wells were emptied gently by pipetting and add 100 µL of biotin-coated antibody work solution. The plates were covered and incubated for 1 hr at 37°C. The wells were discarded after incubation and washed thrice with 1X wash buffer. The 100 µL of SABC working solution was mixed to the decanted wells and incubated at 37°C for 30 min. The suspension was removed and wells were rinsed thrice with saline. To the wells 90 µL of TMB substrate were mixed and incubated the plates in dark at 37°C for 20 min. Finally, 50 µL of stop solution was mixed to the wells and absorbance was taken at 450 nm. Standard curve was plotted with the OD 450 values of standards in Y axis Vs the respective concentration in X axis. The concentrations of samples were measured using the standard curve.

### Quantification of inflammatory cytokines

The inflammatory cytokine levels TNF-α, NFκB, COX-2 and IL-6 were quantified in the control and apocynin treated A549 cells using the ELISA kits procured from Abcam, USA. Same protocol mentioned for quantification of apoptotic proteins were carried for the quantification of inflammatory cytokines also.

### Statistical Analysis

The studies were performed thrice and values obtained was scrutinized with the statistical software GraphPad Prism, version 6.0. The intergroup difference was investigated with one way ANOVA and the intragroup significance was assessed with *post hoc* Tukey's test. The significance was set to be $p<0.05$ and the data were presented as mean±SD.

## RESULTS

### Polyphenol apocynin induced cytotoxicity in A549 cells

Figure 1 illustrates the results obtained from the MTT assay performed with untreated and apocynin treated A549 cells. Apocynin considerably inhibited the viability of A549 in a dose-dependent manner. Control untreated A549 cells were considered as 100% viable and the percentage of cell viability in apocynin treated were calculated accordingly. Minimal dose of 10 µM/mL of apocynin treated cells shown 97±2.2% of cell viability whereas the highest dose treated 100 µM/mL apocynin treated cells shown minimum of 18±1.8% of cell viability. $IC_{50}$ level was fixed at 40 µM/mL of apocynin.

### Polyphenol apocynin induces ROS generation in A549 cells

The levels of reactive oxygen generated in untreated and apocynin treated A549 cells were measured by quantifying the green fluorescence emitted due to DCFH-DA staining. Apocynin treated A549 cells emitted increased level of fluorescence in doses dependent manner indicating the accumulation of ROS. The 40 µM/mL apocynin treated cells shown 29±1.2% of fluorescence whereas it is significantly increased in to 52±2.3% of fluorescence in 60 µM/mL apocynin treated A549 cells (Figure 2).

### Polyphenol apocynin decrease MMP in A549 cells

Mitochondrial activity depends on the mitochondrial membrane potential hence we assessed the role of apocynin on disrupting the MMP potential in A549 cells. Control untreated cells fluorescence was considered to be 100%. The 40 µM/mL apocynin treated cells shown 69±0.8% of fluorescence whereas it is significantly decreased to 53±0.9% of fluorescence in 60 µM/mL apocynin treated A549 cells (Figure 3).

### Polyphenol apocynin induced apoptosis in A549 cells

Figure 4 depicts the representative AO/EtBr-stained images of A549 cells. Control cells shown bright green fluorescence indicating the presence of viable cells. The 40 µM/mL apocynin treated cells displayed greenish orange fluorescence and the 60 µM/mL of apocynin treated cells exhibited bright red orange fluorescence indicating the increased number of apoptotic cells. The 73±1.2% of viable and 25±0.9% of dead cells were detected in 40 µM/mL apocynin treated cells whereas the 60 µM/mL apocynin treatment significantly decreased the viable cell percentage to 30±1.4.

### Polyphenol apocynin induced nuclear damage in A549 cells

Polyphenol apocynin treated A549 cells were subjected to staining with nuclear stain DAPI to assess the nuclear damage induced by apocynin (Figure 5). Both the 40 and 60 µM apocynin treated A549 cells exhibited upsurge in number of fragmented nucleuses containing cells that proves the induction of apoptosis. The results of ImageJ analysis confirms that 38±0.7% and 57±0.9% of cells were apoptotic cells in 40 and 60 µM/mL apocynin treated cells, respectively.

### Polyphenol apocynin induced apoptosis in A549 cells

The apoptosis induction by apocynin in A549 cells were analyzed by estimating the apoptotic proteins Caspase-9, Caspase-3, Bax

and Bcl2 levels using ELISA kits (Figure 6). Both the initiator caspase-9 and executor caspase-3 were significantly augmented in the apocynin treated cells than untreated lung cancer cells. The proapoptotic protein Bax levels were also increased in the apocynin treated A549 cells. The antiapoptotic protein Bcl2 levels were significantly decreased in the apocynin exposed cells than control cells.

## Polyphenol apocynin induced inflammation in A549 cells

Inflammation plays a crucial role in tumor development hence we assessed the levels of inflammatory protein in apocynin treated human lung cancer cells (Figure 7). Apocynin treatment remarkably diminished the levels of proinflammatory cytokines TNFα and NFκB. It also decreased the interleukin-6 and cyclooxygenase levels that confirming the anti-inflammatory potency of apocynin.

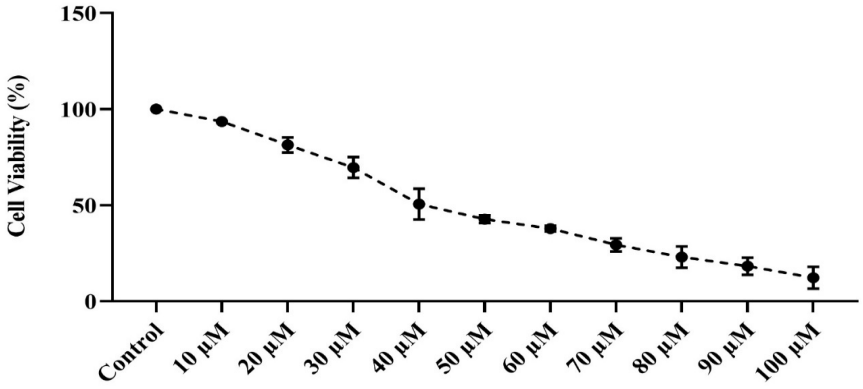


**Figure 1:** Polyphenol apocynin induced cytotoxicity in human lung cancer A549 cells. A549 cells were cultured in DMEM medium and treated with different concentrations of apocynin 10-100 µM/mL for 24 hr. Subjected to MTT assay in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

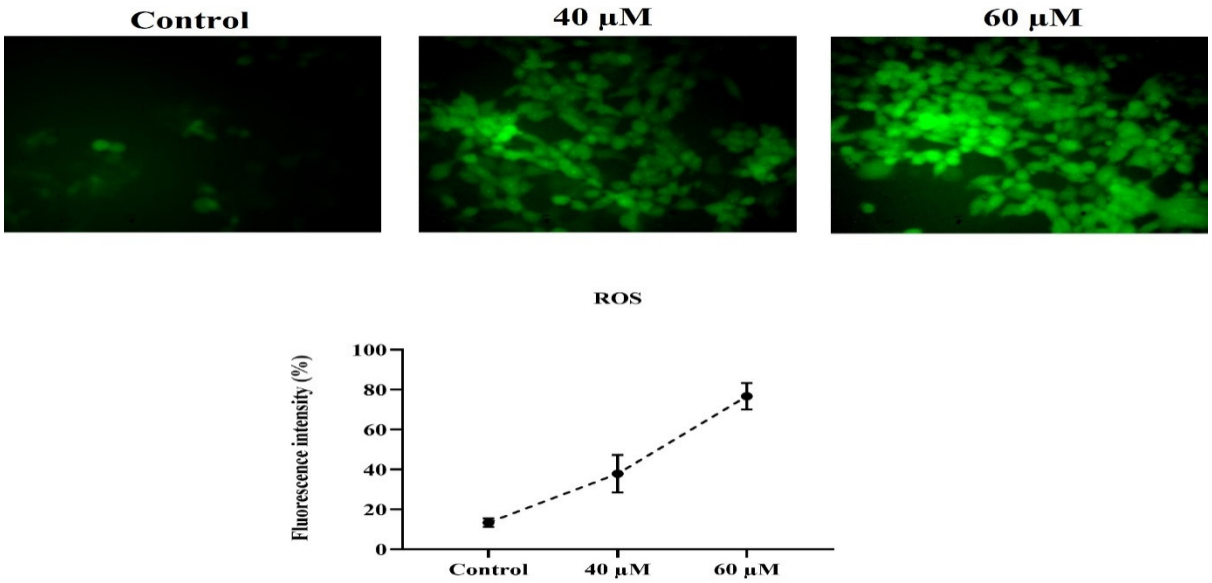


**Figure 2:** Polyphenol apocynin induces ROS generation in human lung cancer A549 cells. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to 2,7-Dichlorodihydrofluorescein Diactate (DCFH-DA) staining. The images were assessed with ImageJ software. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

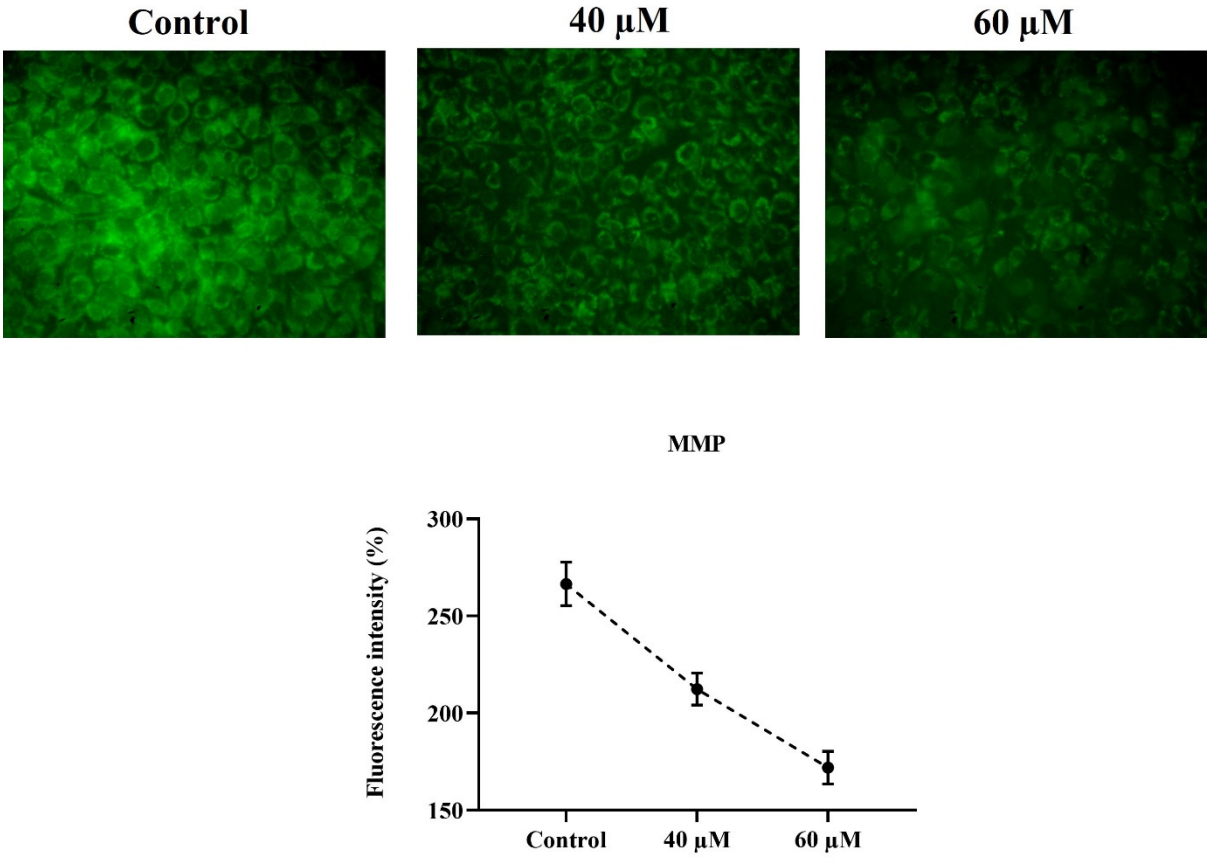


**Figure 3:** Polyphenol apocynin decrease mitochondria membrane potential in human lung cancer A549 cells. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to Rhodamine 123 staining. The images were assessed with ImageJ software. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant

## DISCUSSION

In the current study, we attempted to analyze the potency of the phytochemical apocynin to induce apoptosis in lung cancer cells. Lung cancer is one of the deadliest diseases which were ranked to second in cancer related global mortalities lack efficient treatment. The major drawback of treating lung cancer is lack of diagnosis at early stages.[3] Most of lung cancer patients were diagnosed at the metastasis stage in which the traditional treatments such as surgery, radiotherapy and chemotherapy are in effective.[4] Even though some chemotherapeutic drugs are available to treat lung cancer it induces numerous side effects which is worse than the cancer induced health morbidities.

Apocynin is polyphenolic compound isolated from *Picrorhiza kurroa* perennial plant present in Himalayas.[16] The extracts of this medicinal plant were used in the ancient ayurvedic medicine to treat diseases related to liver, lung, heart and joints. Apocynin is effective Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-oxidase inhibitor.[17-20] Therefore it is proven to be potent against inflammatory diseases such arthritis, neurodegenerative diseases, stroke, asthma, inflammatory bowel disease, Crohn disease, colitis etc.,[21] Few studies have reported apocynin inhibits the cancer cell progression in prostate,[22] breast[23] and pancreatic cancer cells.[24] We analyzed the role apocynin against lung cancer cells.

The impact of apocynin on cell survival of A549 cells were examined by treating the cells with different concentration of apocynin for 24 hr and subjected to MTT assay. At 24 hr incubation apocynin effectively inhibited the survival of lung cancer and induced 50% of cell death at the dose of 40 µM/mL concentration. Hence for assessment of the anticancer property we treated the A549 cells with a dose of 40 and 60 µM/mL which is lesser than the $IC_{50}$ dose of apocynin on A549 cells.

ROS plays a vital role in cellular homeostasis maintenance and the dysregulation in ROS and antioxidants leads to cancer cell proliferation and progression. ROS is a double-edged sword not only induces the tumor cell development but in certain threshold it also triggers apoptosis in tumor cells. Hence most of the anticancer drugs are designed to enhance the ROS in cancer cells thereby inducing cancer cell apoptosis.[25-28] It often impairs the antioxidant system in cancer cells which aids the drug resistance

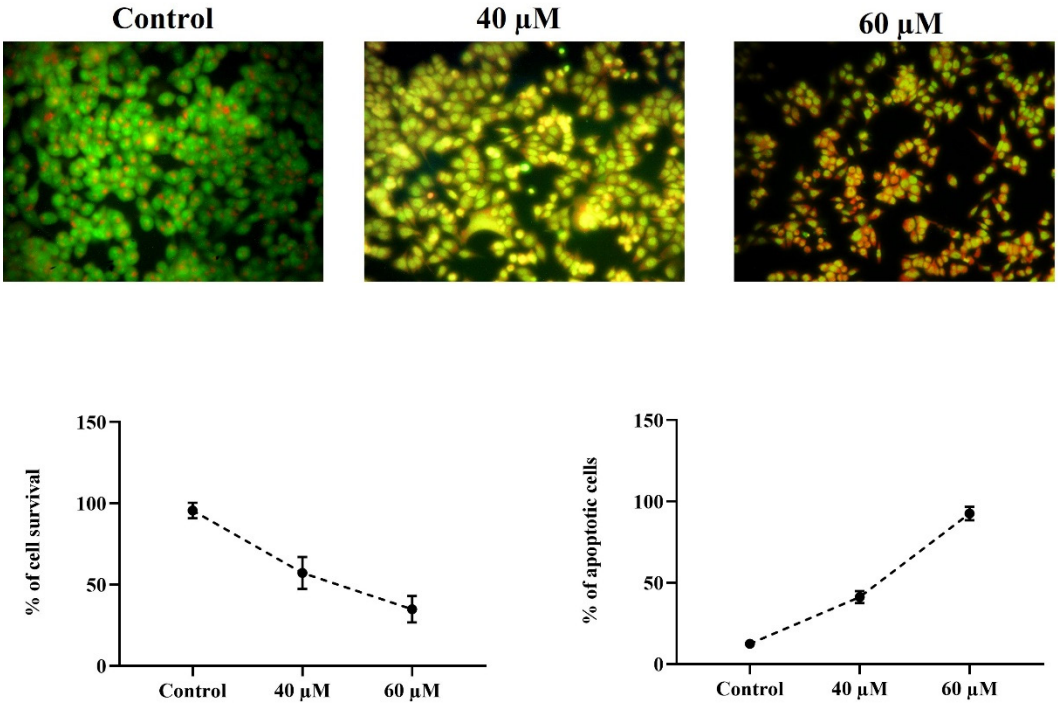


**Figure 4:** Polyphenol apocynin induced cell death in human lung cancer A549 cells. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to dual staining with acridine orange and ethidium bromide mixture (1:1). The images were assessed with ImageJ software. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

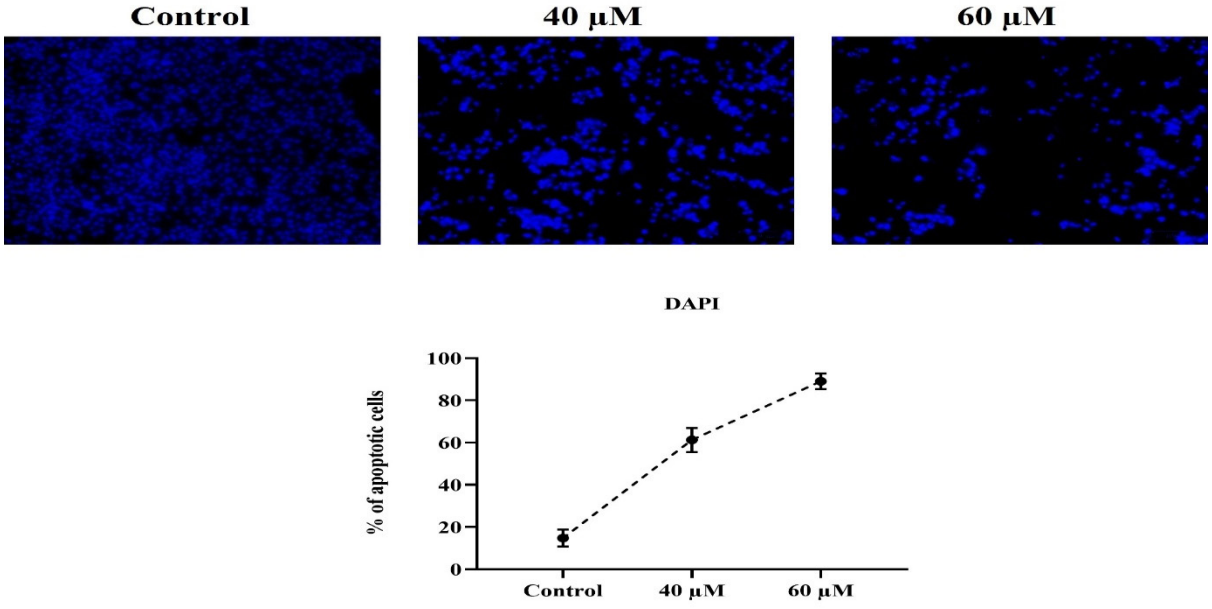


**Figure 5:** Polyphenol apocynin induced nuclear damage in human lung cancer A549 cells. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to DAPI staining. The images were assessed with ImageJ software. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

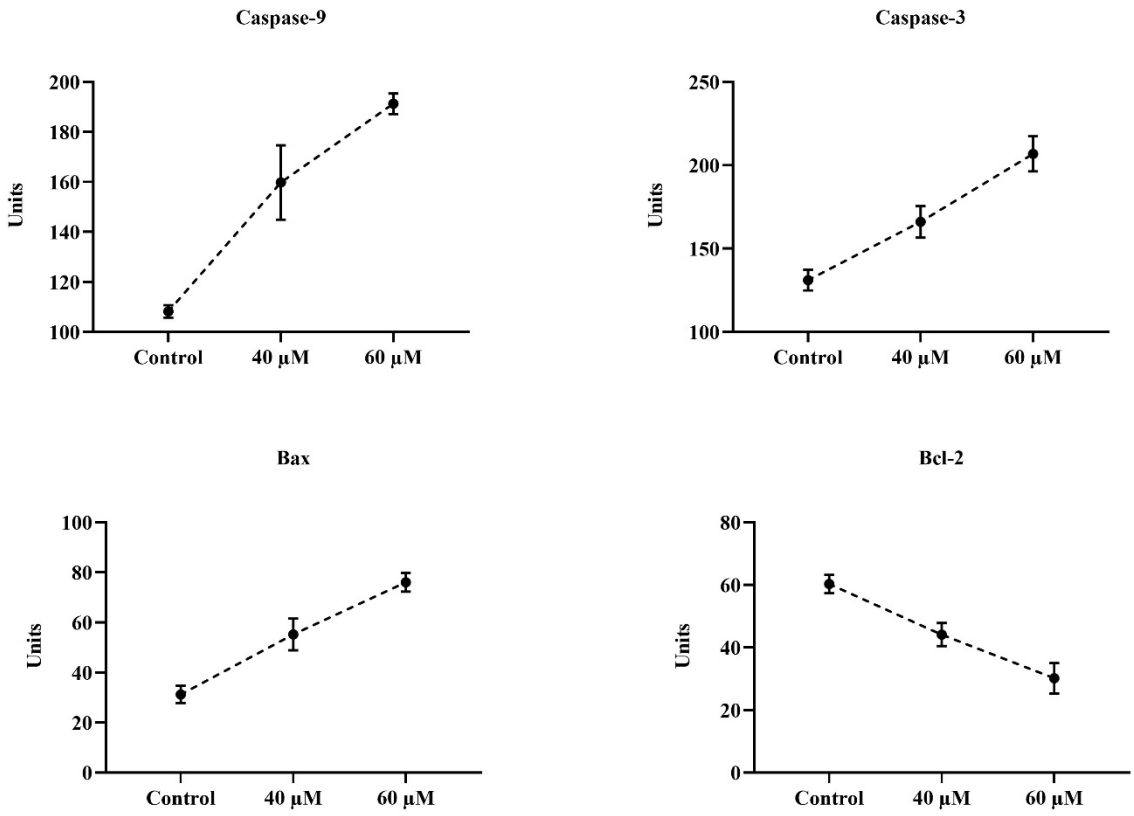


**Figure 6:** Polyphenol apocynin induced apotosis in human lung cancer A549 cells. A) Caspase 9, B) Caspase 3 C) Bax D) Bcl2. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to quantification of apoptotic proteins using ELISA kits. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

in cancer cells. The presence of increased antioxidants like GSH as glutathione impairs the drug potency of anticancer drugs such taxol. Arsenic trioxide etc., Anticancer drug Procarbazine induces apoptosis in Hodgkin's lymphoma via ROS induced oxidative nuclear damage.[29] Doxorubicin a potent anticancer prescribed to treat various cancers such as breast, Kaposi's sarcoma, leukemia, bladder stimulates the Fenton reaction thereby induces the generation of hydroxyl radicals causing oxidative stress induced apoptosis.[30,31] Hence we assessed the efficacy of apocynin to induce ROS generation in A549 cells. Apocynin significantly increased the levels of ROS in A549 cells.

Increase in ROS acts on sphingomyelinase induces the synthesis of ceramide which binds to the cell membrane death receptors of cancer cells causing apoptosis. Lymphoma cells induced with UV radiation activates this pathway thereby collapses mitochondria and leads to apoptosis.[32] Drugs such as gamitrinib induces mitochondrial damage in prostate[33] while quinone derivative drug ARQ501 disrupts electron transport chain thereby leads to apoptosis in pancreatic cancers.[34] Apocynin treatment also disrupted the MMP in A549 cells which was evidenced by Rhodamine 123 staining results. The disruption of MMP may because of the increase in ROS induced by the apocynin treatment.

Phytochemicals like resveratrol, thymoquinone, ginsenosides acts as prooxidants induces generation and generation of ROS in mitochondria of cancer cells such as PC-3, MCF-7, HepG2 thereby induces apoptosis.[35,36] ROS triggers various signaling pathways activates Akt which further activates apoptotic protein Bax. Activated Bax disrupts the MMP and release cytochrome-C into the cytosol.[37] In cytosol cytochrome activates procaspase -9 and forms apoptosomes. It in turn activates effector caspase 3 which cleaves the cellular protein causing apoptosis.[38] In our study apocynin treatment significantly increased Bax and also both the initiator caspase-9 and the effector Caspase 3 which confirms the activation of apoptotic signaling pathway. The activation of these apoptotic proteins may be due to the increased ROS accumulation which was confirmed with DCFH-DA staining results. Further the results of DAPI and AO/EtBr staining proves the initiation of apoptosis in A549 cells by apocynin.

Inflammation is another major entity which regulates the cancer cell growth and progression. The levels of inflammatory cytokines in the tumor cells determines the transformation of

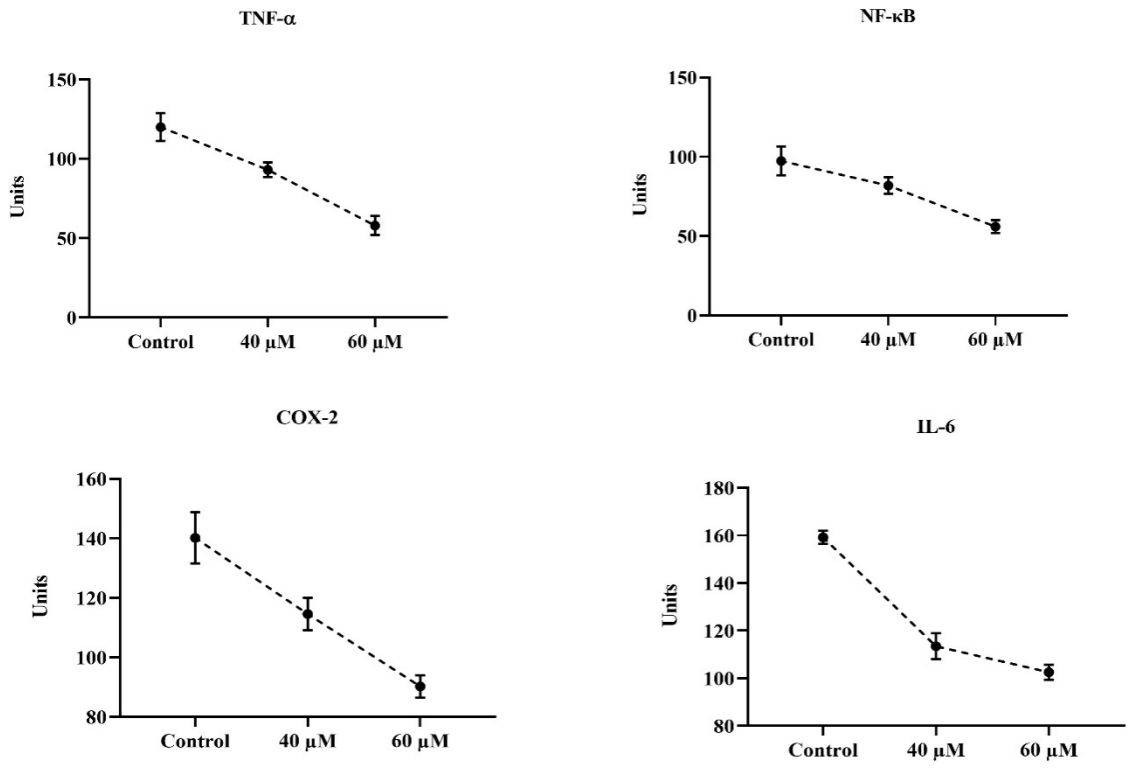


**Figure 7:** Polyphenol apocynin induced inflammation in human lung cancer A549 cells. A) Tumor necrosis factor alpha-TNFα B) Nuclear factor kappa B-NFκB C) Cyclooxygenase -2 COX-2 D) Interleukin-6 IL-6. A549 cells were cultured in DMEM medium and treated with two different concentrations of 40 and 60 µM/mL apocynin for 24 hr. Apocynin treated cells were subjected to quantification of inflammatory cytokines using ELISA kits. Experiments were done in triplicates and the results were assessed with one way ANOVA followed by *post hoc* Tukey's test. Data were presented as mean±SD, $p<0.05$ considered to be statistically significant.

malignant cells and also induces antitumor response.[39-41] ROS is key intermediator between the inflammatory cytokines and cancer cells it regulates the levels and activation of inflammatory cytokines.[42,43] ROS accumulation, Chronic inflammation and cancer cell progression are interlinked signaling pathways. ROS promotes TNF-α and NFκB levels, the activated NFκB is the prime inducer of drug resistance for various mostly prescribed anticancer drugs such as tamoxifen, cisplatin, daunorubicin, 5-fluorouracil etc., The activation of NF-κB up regulates cell survival proteins COX-2, Bcl2, Akt, cyclin D1, surviving.[44-46] Therefore anticancer drugs were targeted to increase the ROS production and decrease the inflammatory cytokines thereby inhibit the cancer proliferation and metastasis.[47,48] In our work also apocynin remarkably augmented the ROS accumulation and decreased the TNF-α, NFκB, COX-2 and IL-6 that confirms it may be a talented anticancer candidate to treat human lung cancer.

## CONCLUSION

To conclude our findings suggests phytochemical apocynin significantly increased the ROS generation in A549 cells which in turn disrupted the mitochondrial membrane potential and triggered the apoptosis signaling molecules. It also effectively diminished the proinflammatory cytokines in A549. Apocynin is a talented anticancer and anti-inflammatory drug which possess the potency to be developed as anticancer agent to treat human lung cancer in future.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**NSCLC:** Non-small cell lung cancer; **DMEM:** Dulbecco's Modified Eagle's Medium; **FBS:** Fetal bovine serum; **DMSO:** Dimethylsulfoxide; **DCFH-DA:** 2,7-dichlorodihydrofluorescein diactate; **MMP:** Mitochondrial Membrane Potential.

## SUMMARY

Apocynin is one such phenolic compound extracted from the *Apocynum cannabinum* and *Picrorhiza kurroa* roots which are numerous studies have proven the efficacy of apocynin on treating various disease. Lung cancer, widely considered the leading cause of cancer-related fatalities globally, ranks fourth in terms of its occurrence among all types of malignant tumors. apocynin significantly increased the ROS generation in A549 cells which in turn disrupted the mitochondrial membrane potential and triggered the apoptosis signaling molecule. Apocynin remarkably augmented the ROS accumulation and decreased the TNF-α, NFκB, COX-2 and IL-6 that confirms it may be a talented anticancer candidate to treat human lung cancer.

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