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Targeting NF-κB mediated cell signaling pathway and inflammatory mediators by 1,2-diazole in A549 cells *in vitro*

Venugopal Vinod Prabhu[a,*], Perumal Elangovan[a], Sivasithambaram Niranjali Devaraj[a], Kunnathur Murugesan Sakthivel[b]

[a] Department of Biochemistry, University of Madras, Guindy Campus, Chennai, 600025, Tamil Nadu, India
[b] Department of Biochemistry, PSG College of Arts and Science, Coimbatore, 641014, Tamil Nadu, India

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ABSTRACT

Lung cancer is the leading cause of cancer deaths globally. The objective of this study was to investigate the effect of 1,2-diazole (pyrazole) as an anti-cancer drug on human non-small cell lung carcinoma A549 cells. We attempt to examine the expression level of pro-inflammatory proteins such as TNF-α, NF-κB-p65, MMP-2 and E-Cadherin which are commonly associated with an inflammatory response in epithelial cells and apoptosis in A549 cells. The LPS-induced cytokines and inflammatory mediators include TNF-α, IL-6, iNOS and COX-2 levels in A549 cells and the effect of pyrazole was studied. The present study reveals that, pyrazole inhibits A549 cells by suppressing TNF-α induced MMP-2 expression, thereby inhibiting the nuclear translocation of NF-κB-p65. Pyrazole significantly up-regulate the E-cadherin level and down-regulated MMP-2 expression that could probably preventing A549 cancer cells to invade. The study further substantiated the anti-cancer property of pyrazole by regulating the above mentioned level of LPS-induced cytokines and inflammatory mediators. The observations of the present study open a possibility for the development of an effective therapeutic agent that targets inflammatory and signaling pathway mediators to challenge human non-small cell lung carcinoma.

1. Introduction

Lung cancer is a foremost cause of cancer deaths around the globe and the survival rate is reported to be about 14 % [1,2]. The increase in the mortality rate is directly linked to irregular modifications in the lung cancer cells that may be a consequence of aggressive invasion and metastasis [3]. Among the categories of lung cancer, about 80 % are belongs to non-small cell lung carcinoma (NSCLC) [4]. Surgical resection, chemotherapy and radiation therapies are often insufficient since these tumor cells migrate and metastasize [5–8]. Recent findings have led to targeted therapeutic strategies. Inhibitor that target signaling and apoptotic pathways and regulators of cell cycle which could serve as potential treatment options/therapy for lung cancer [4]. Concurrently, medications from natural origin are also efficient in treatment of lung cancer with restricted adverse effects [8,9]. The lung epithelium which is involved in the inflammatory process either directly or indirectly by interaction with various mediators released from alveolar macrophage results in the signaling of an inflammatory response in the epithelial cells through which transcription factor gets activated. This leads to production of many pro-inflammatory proteins by epithelial cells, including cytokines in the lung that effect on the body as a whole [10–12].

Plant derived secondary metabolites such as alkaloids, flavonoids, phenolics, steroids and terpenoids have been reported to possess significant pharmacological and toxicological activity [9,13–15]. It is well known that, natural compounds obstruct several signaling pathways and also have low toxicity to normal tissues [16]. Numerous studies reported that compounds derived from plant based marine/aquatic environment had distinguishing structural features with inhibitory activity on multiple signaling pathways which was not present in terrestrial based plant derived compounds [8,13]. Wedelolactone, parthenolide and honokiol, are selective natural chemopreventive agents from the marine environment that inhibited IκB kinase, suppressed both tumor necrosis factor (TNF-α)-induced IκB phosphorylation along with NF-κB phosphorylation [17,18].

We have previously reported that marine mangrove, *Rhizophora apiculata* (*R.apiculata*) contains high level of 1,2- diazole (pyrazole) in the crude methanolic plant extract which showed remarkable anti-inflammatory and nephroprotectant properties. Pyrazole exhibited its anti-cancer role by initiating apoptosis by regulating

* Corresponding author.
E-mail address: vinodprabu3k@gmail.com (V. Vinod Prabhu).

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Epidermal Growth Factor Receptor (EGFR), Cyclin-dependent kinase 2 (CDK-2) and B-cell lymphoma-2 (Bcl-2) mediated signaling pathways in NSCLC [8,19–21]. The derivatives of pyrazole (shown in Fig. 1) are a class of compounds having ring C3N2 with adjacent nitrogen atoms. The pyrazole derivatives were reported to be first extracted from watermelon seeds and they were classified as alkaloid group. Pyrazole and its derivatives have been reported to have pharmacologically active properties such as anti-microbial, anti-inflammatory and anti-cancer activities [9,22–25]. Hence, based on our earlier report on pyrazole's anti-cancer activity, we further investigated its detailed mechanism of action by examining the expression level of inflammation responsive mediators include TNF-α, NF-κB-p65, matrix metalloproteinase 2 (MMP-2) and E-cadherin that are commonly associated with the inflammatory response in epithelial cells, cell-cell adhesion process and apoptosis mechanism.

2. Materials and methods

2.1. Chemicals and media

Pyrazole was obtained from Sigma Aldrich, India. Ham's F-12 K (Kaighn's) Culture Medium, Trypsin, Fetal Bovine Serum (FBS), Bovine Serum Albumin (BSA), Antibiotic-actinomycotic solution were obtained from Hi-Media laboratories, India. Monoclonal antibodies (rabbit) such as MMP-2 (D8N9Y), NF-κB-p65 (D14E12) XpR, E-Cadherin (24E10), TNF-alpha, β-actin and HRP conjugated secondary antibodies (anti-rabbit) were purchased from Cell Signaling Technology (USA). Desalted & Hi-Pure Oligos include MMP-2, NF-κB-p65, E-Cadherin, TNF-α and β-actin were obtained from Eurofins Genomics (Bengaluru, India). Cytokine ELISA kits include TNF-α and IL-6 from Koma Biotech (Korea), iNOS and COX-2 from USCN Life science (China) were purchased.

2.2. Cell culture and drug preparation

A549 cell line (Stock culture) was procured from National Centre for Cell Science (NCCS), Pune, India. It was allowed to grown at 37 °C temp with 5% CO2 and maintained in Ham's F-12 which contains 10 % FBS. Solution of pyrazole (75μM) was prepared in distilled water and diluted in Ham's F-12 medium, as required. The final concentration of Ham's F-12 medium was minimalized to 0.1 % and this concentration was found to be not toxic to the survival of cells. The non-toxic concentration of pyrazole drug at (IC50 value-75μM) was selected based on *in-vitro* MTT assay reported in our earlier study [8].

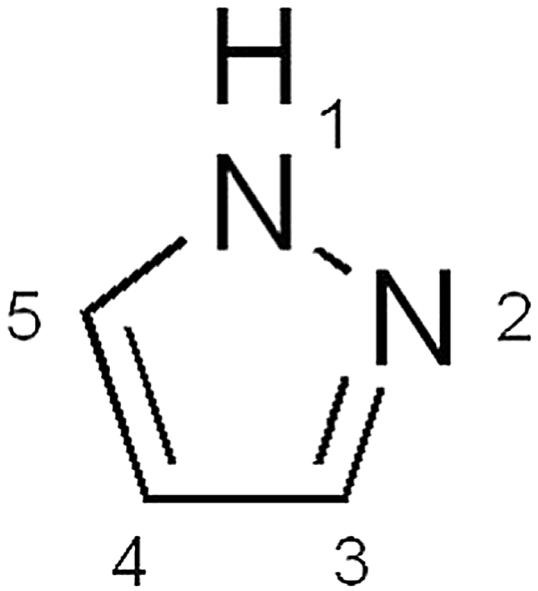

Fig. 1. Representation of Structure of 1,2-diazole (pyrazole).

2.3. Reverse transcription-polymerase chain reaction analysis (RT-PCR analysis)

The total RNA was extracted from untreated A549 cell line (control) and A549 cells treated with pyrazole as described previously [26,27]. The RNA concentration was determined at optimal density (OD) 260 nm [27]. RNA samples (calculated volume) were transcribed into cDNA by means of standard protocols purchased from Thermo Fisher Scientific India Ltd. PCR was carried out using the procured oligonucleotide sequences and the amplification was done as per the condition provided in Table 1. Beta-actin used as loading control for all the experiments in the present study.

2.4. Western blotting

The protein lysate (~20–40 μg) was separated by SDS-PAGE (10% gel) and transferred to PVDF (poly vinylidene difluoride) membrane (Bio-Rad) for 90 min. at 100 mA. Then, PVDF membranes was incubated overnight with MMP-2, NF-κB-p65, E-Cadherin, TNF-α [rabbit mab (1:1000 dilution) respectively] and β-actin mab (1:1000 dilution) in 5% BSA solution. The steps followed by primary antibody incubation, they were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibodies (1:5000 dilution) for about 2 h at room temperature. The immunoreactive labelled proteins were detected using the Enhanced chemiluminescent (ECL) system. Beta-actin was used as loading control. The intensities of the protein bands obtained were examined using ImageJ software (NIH, USA) [27–29].

2.5. Quantification of Cytokines and Inflammatory mediators in lipopolysaccharide (LPS) induced A549 cells

The effect of pyrazole (75μM) on Lipopolysaccharides (LPS)-induced cytokine production of TNF-α, IL-6, iNOS and COX-2 levels were measured by ELISA method using culture supernatants using a microplate reader. For the estimation of above mentioned cytokine levels, A549 cells were maintained in Ham's F-12 supplemented with 10 % FBS at 37 °C temp and 5% CO_2. Cells (100 μl) were seeded (5×10^5 cells/well) in to 6-well plate in culture medium (3 mL) for 24 h. Cells were changed to 3 mL of serum-free medium 24 h before the assay and exposed to 75μM concentration of pyrazole (50 μl). One hour later, LPS was added to appropriate wells at a concentration (5 μg/mL) and incubated for another 24 h. Control, untreated cells were incubated with 50 μl of growth medium (vehicle). Finally, the plates were centrifuged (1200 RPM) for ~5 min to remove all cell debris and the culture supernatants (1 mL) were harvested and analyzed for the secreted levels of above mentioned proteins according to standard protocols as per manufacturer's instructions. The amount of produced proteins were calculated using standard curve constructed by serial dilutions of standards (cytokines) provided with the kit. The data were represented as Mean ± SD. All experiments were performed as triplicates [30].

2.6. DNA fragmentation analysis

Extraction of DNA and agarose gel electrophoresis were performed by using standard protocols as mentioned earlier in the previous study

Table 1
List of genes, and their primers with their product size.

Name of the gene	Primers 5′-3′	Product size
TNF-α	**Forward primer:** CTCGAACCCCGAGTGACAAG **Reverse primer:** TGAGGTACAGGCCCTCTGAT	**159**
NF-κB-p65	**Forward primer:** GCGAGAGGAGCACAGATACC **Reverse primer:** AGGGGTTGTTGTTGGTCTGG	**279**
E-Cadherin	**Forward primer:** GGCTGGACCGAGAGAGTTTC **Reverse primer:** TCAAAATCCAAGCCCGTGGTG	**157**
MMP2	**Forward primer:** CCTCCTAGTAGTACCGCTGC **Reverse primer:** ATTGCTCTCTCGCGATCTGG	**111**

[31]. Briefly, 1 × 10^5 A549 cells/well were seeded in Hams F-12 medium and incubated at 37 °C in 5% CO2. Then, incomplete media with pyrazole (75μM) were incubated for 24 and 48 h separately. Post 24 and 48 h of treatment, the cells were extracted and then washed with Phosphate Buffer Saline and transfer to micro-centrifuge tubes (1.5 mL) which contains 20 μL of lysis buffer. To the tubes, 10 μL of RNase Cocktail (RNase A and T1 at 500 units/mL and 20,000 units/mL, respectively) were added and incubate for ∼30−120 min at 37 °C. 10 μL of proteinase K (Concentration-20 mg/mL) were added, and incubate at 50 °C for 90 min. DNA samples were loaded in to 1.5 % agarose gel along with 100-bp size ladder. DNA ladders are finally visualized and documented.

2.7. Statistical analysis

Statistical analysis was performed using SPSS (Version 11.5). Values were represented as Mean ± SD. ANOVA (One-way Analysis of Variance) test was used to evaluate the significance between control and treated groups. The statistical significance (i.e., *p-value*) was determined at a $^*p < 0.05$ level.

3. Results

3.1. Pyrazole blocks TNF-α induced NF-kB activation pathway in A549 cells

The gene expression which are linked with the inflammatory proteins in A549 cells were studied by RT-PCR. The mRNA expression of the cytokine TNF-α showed strikingly significant difference in the two different groups investigated which included the untreated control A549 cells and pyrazole treated. Relative expression level of TNF-α mRNA of A549 cells was found to be greatly downregulated in 24th hour and 48th hour treatment, when in comparison with the untreated control A549 as depicted in Fig. 2 and 3. The mRNA expression of cell survival signaling protein NF-κB-p65 in A549 cells was studied and the mRNA expression of NF-κB-p65 showed a pronounced difference between the control untreated A459 and pyrazole treated cells. The relative mRNA expression levels of NF-κB- p65 of A549 cells was differentially downregulated in 24th and 48th hour treatment when compared with control cells (Figs. 2 and 3).

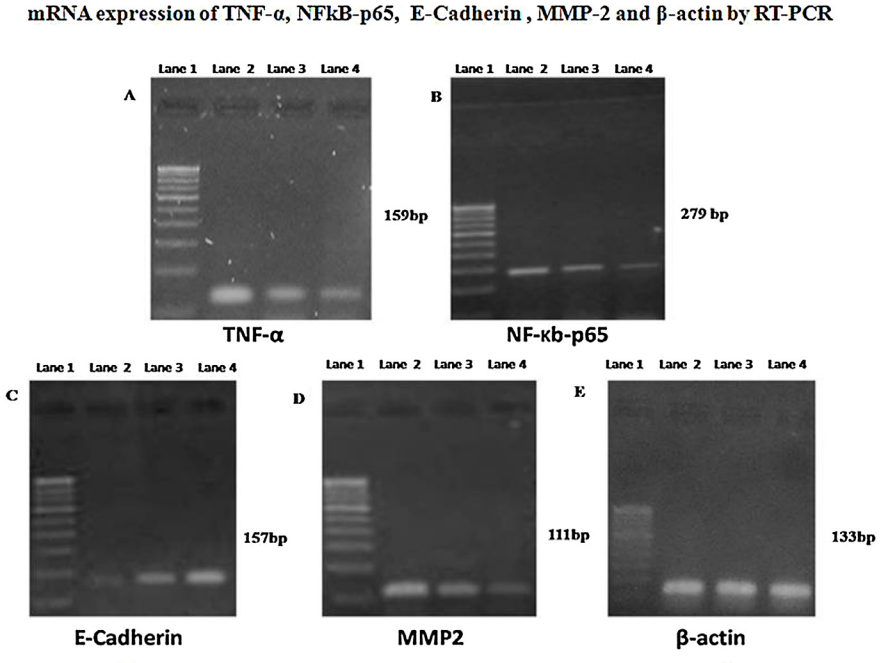

Fig. 2. RT-PCR analysis of mRNA expression: (A) TNF-α, (B) NF-κB-p65, (C) E-Cadherin, (D) MMP-2 and (E) β-actin are presented as compared with A549 control (untreated) cells. Lane 1- DNA Ladder, Lane 2- A549 control (untreated) cells, Lane 3 & 4 - Pyrazole treated A549 cells (24 & 48 h respectively).

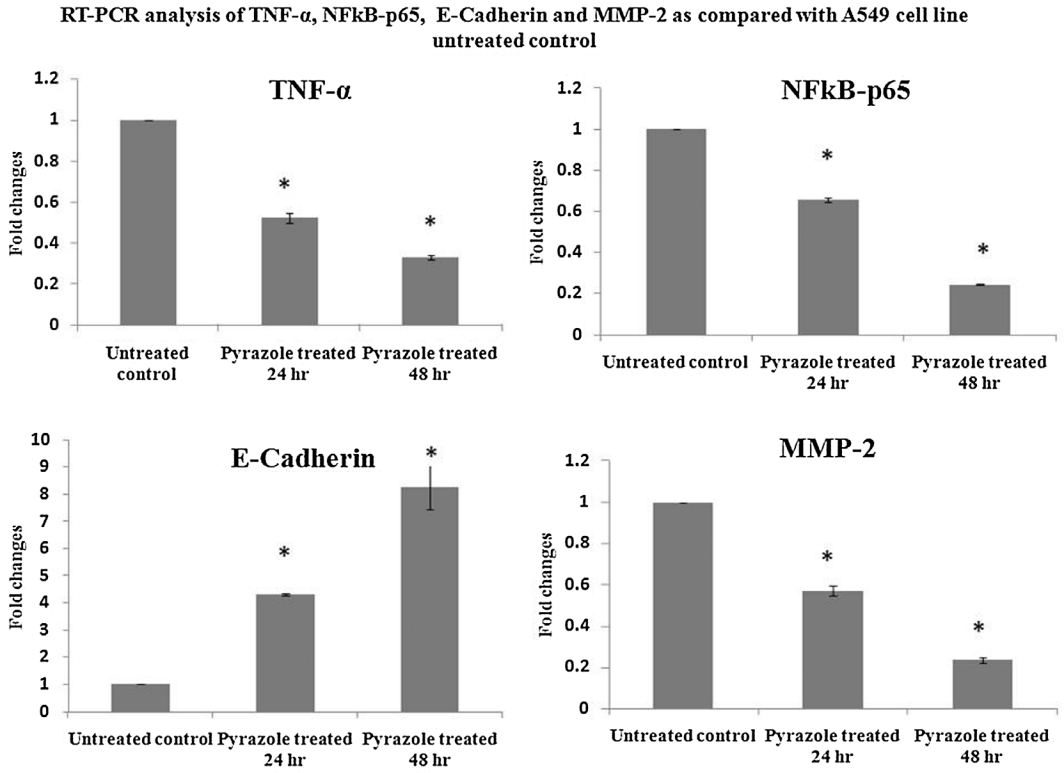

Fig. 3. Densitometric values of mRNA expression: The densitometric values of mRNA expression of TNF-α, NF-κB-p65, E-Cadherin, and MMP-2 normalized with β-actin are presented as compared with untreated A549 control cells. Values are expressed as Mean ± SD (n = 3). *$p < 0.05$, the p-value is considered as significant from untreated control.

3.2. Pyrazole suppresses the invasion capacity of A549 cells mediated by E-Cadherin-MMP mediated signaling pathway

Loss of expression of E-cadherin levels and overexpression of MMPs are characterized as predominant features of invasion ability, therefore, the ability of pyrazole to suppress the invasive ability of A549 cells was investigated. The gene expression levels of E-Cadherin was carried out by RT-PCR analysis which shows significantly increased levels in pyrazole treated when compared with untreated A549 cells (Fig. 2 and 3).

In addition, we also examined the expression levels of MMP-2 in the pyrazole treated A549 cells by RT-PCR. The MMP-2 expression showed highlighting difference between the two groups investigated. The relative gene expression level of MMP-2 was downregulated in both the time periods investigated in the pyrazole treated groups when compared with A549 control cells as shown in Figs. 2 and 3. These results indicated that pyrazole inhibited A549 cells through E-Cadherin mediated MMP-2 down regulation.

3.3. Differential expression of inflammatory proteins and tumor suppressor gene upon treatment with pyrazole in A549 cells

The molecular mechanism of action underlying the inhibitory effect of pyrazole on pro-inflammatory signaling and invasion of A549 cells were examined. The study found that TNF-α, and NF-κB-p65 protein expressions were significantly down regulated in both the time points 24 and 48th hour pyrazole treated A549 cells when compared with untreated A549 control cells. The protein expression of E- cadherin as determined by western blot analysis was upregulated in pyrazole treated A549 cells when compared to untreated A549 control cells. The effect of pyrazole on E-cadherin expression was substantiated by the downregulation of MMP-2 protein expression in treated A549 cells at 24th and 48th hour when compared to A549 (untreated) control cells. Altogether, the results of these experiments indicate that TNF-α and NF-κB-p65 signaling pathway and metastatic potential could be blocked by pyrazole treatment in

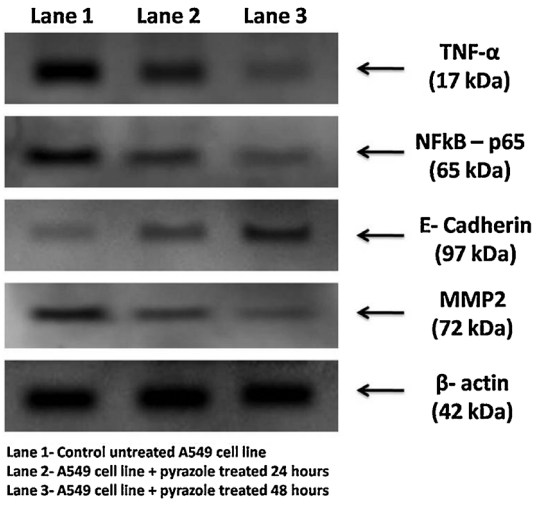

Fig. 4. Effects of pyrazole on the expressions of TNF-α, NF-κB-p65, E-Cadherin, MMP-2 and β-actin in A549 cells. Cells were treated with pyrazole (75 μM) and the expressions of TNF-α, NF-κB-p65, E-Cadherin, MMP-2 and β-actin were determined by western blot compared with A549 control (untreated) cells. Lane 1- Untreated A549 control cells, Lane 2 & 3 - pyrazole treated A549 cells (24 & 48 h respectively).

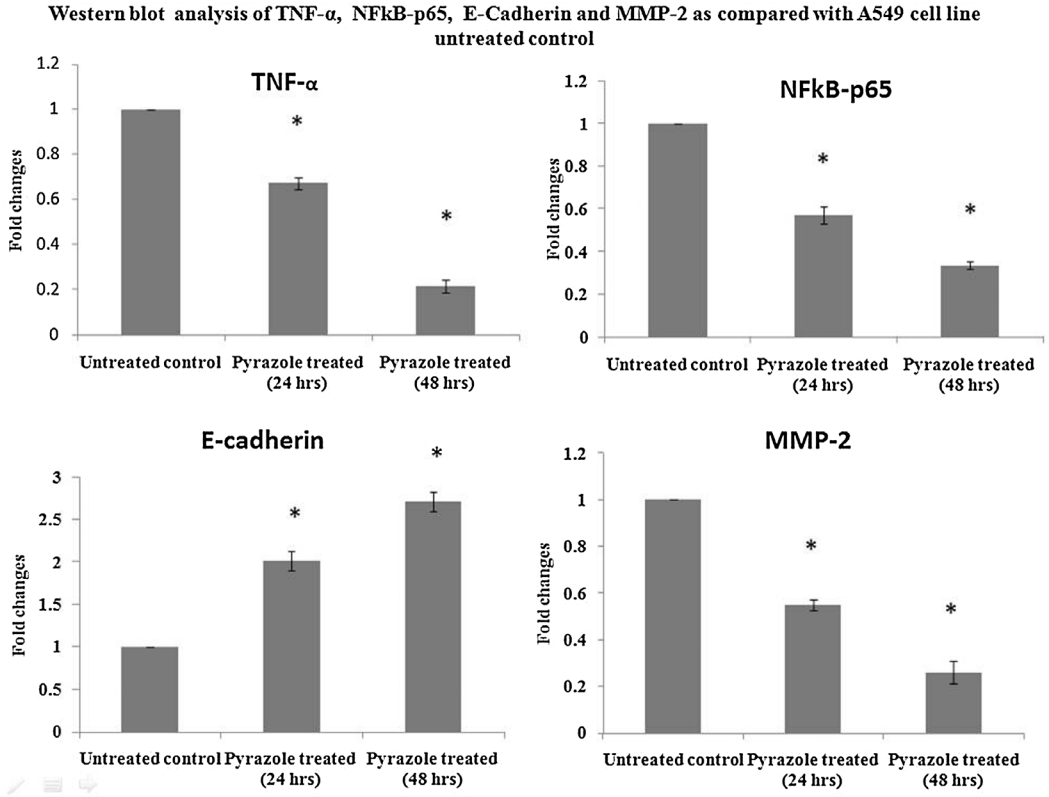

Fig. 5. Densitometric values of the expression of TNF-α, NF-κB-p65, E-Cadherin, and MMP-2 were represented as compared with untreated control A549 cells. Values are expressed as Mean ± SD (n = 3). (*$p < 0.05$) the p-value is considered as significant from untreated control.

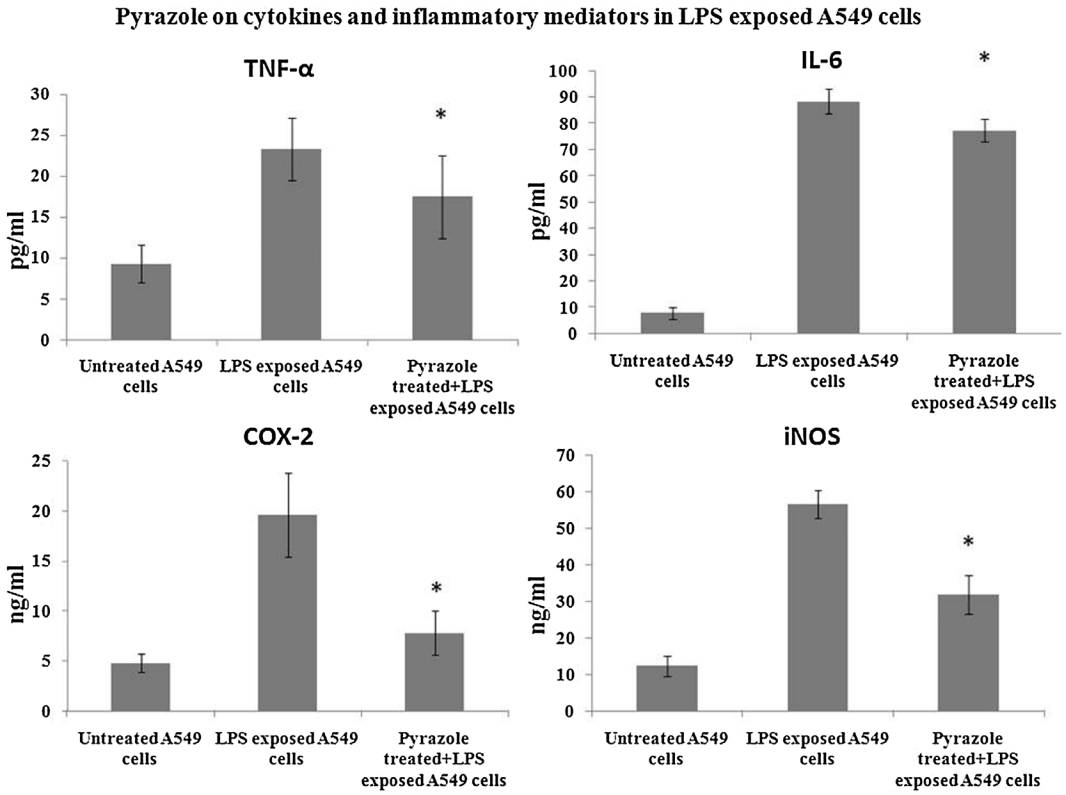

Fig. 6. Effect of pyrazole on cytokines and inflammatory mediators in lipopolysaccharide (LPS) exposed A549 cells: Densitometric values of the level of TNF-α, IL-6, iNOS and COX-2 in LPS exposed A549 cells treated with pyrazole at 75 μM concentrations for 24 h and 48 h are resented as compared with untreated control cells and LPS alone exposed A549 cells. Values are expressed as Mean ± SD (n = 3). *$p < 0.05$, the p-value is considered as significant from untreated control.

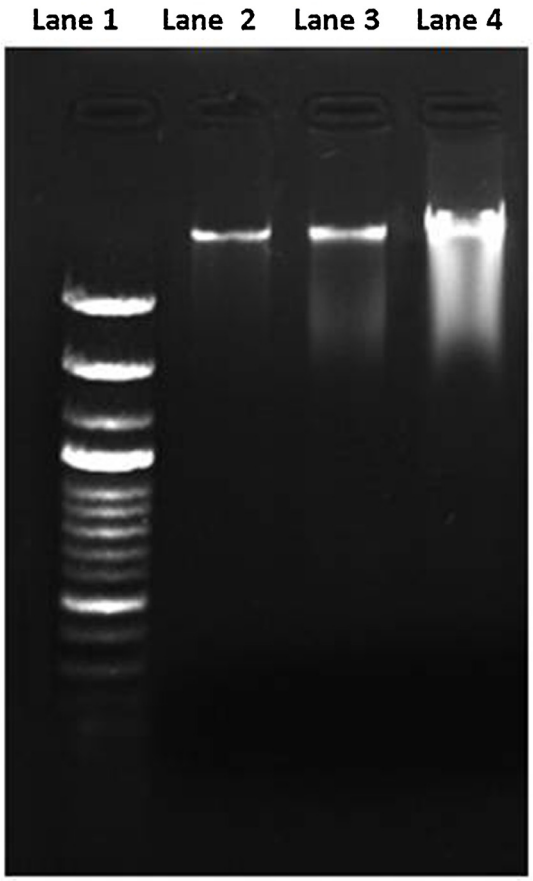

Fig. 7. DNA fragmentation using agarose gel electrophoresis of DNA extracted from A549 cells treated with pyrazole (75 μM) for 24 and 48 h. Lane 1- DNA marker, Lane 2- A549 cells, Lane 3 & 4 - pyrazole treated A549 cells (24 & 48 h respectively).

human NSCLC A549 cells resulting in the suppression of lung cancer invasion as well as metastasis. The effect of pyrazole on the protein expressions of TNF-α, NF-κB-p65, E-Cadherin, MMP-2 and β-actin in A549 cells and their respective densitometric values are shown in Figs. 4 and 5.

3.4. Pyrazole regulates the level of cytokines and inflammatory mediators in A549 cells treated with lipopolysaccharide (LPS)

The LPS-induced inflammatory and cytokine mediators like IL-6, iNOS, TNF-α, and COX-2 levels in A549 cells and the effect of pyrazole was studied. Treatment with pyrazole in LPS exposed A549 cells significantly decreased the TNF-α level in the cells (17.4 ± 5.1 pg/mL) when compared with the LPS alone exposed A549 cells (23.2 ± 3.8 pg/mL). Treatment with pyrazole in LPS exposed A549 cells significantly decreased the IL-6 level in the cells (77.3 ± 4.3 pg/mL) when compared with the LPS alone exposed A549 cells (88.3 ± 4.6 pg/mL). The levels of iNOS were also significantly reduced in pyrazole treated- LPS exposed A549 cells (31.8 ± 5.2 ng/mL) when compared with the LPS alone exposed A549 cells (56.4 ± 3.9 ng/mL). The key inflammatory cytokine COX-2 levels were also significantly reduced in pyrazole treated LPS exposed A549 cells (7.8 ± 2.2 ng/mL) when compared with LPS alone exposed A549 cells (19.6 ± 4.2 ng/mL) as shown in Fig. 6.

3.5. Pyrazole induced apoptosis of A549 cells

The A549 cells were treated with 75 μM of pyrazole for 24th and 48th hour produced DNA fragmentation as demonstrated by smear like appearance in the agarose gel, however ated control A549 cells showed intact genomic DNA. The striking characteristics of apoptotic cells include chromatin condensation and denaturation of the cells. The results demonstrated that pyrazole treated group resulted in the apoptosis of cancer cell lines which demonstrated its potential role as anticancer agent (Figs. 7 and 8).

4. Discussion

Lung cancer remains one of the primary concerns contributing to the deaths around the world. [32] Inflammation is a key factor that has been linked with tumor invasion, metastasis and cancer mortality [33,34]. Inflammatory cytokines activate a cell signaling pathways that promotes cancer development, therefore targeted inhibition of these signaling pathways is required to initiate apoptosis and to retard invasion and metastasis [16,35]. Hence, an effective, precise treatment approach is necessary to reduce lung cancer mortality. In an earlier study, we had established that pyrazole had a predominant inhibitory role on cell proliferation and initiated apoptosis in A549 cells by regulation of different signaling pathways like EGFR, Bcl-2 and CDK-2 [8]. Therefore, in the current study we further demonstrated the regulatory function of pyrazole on the expression of pro-inflammatory proteins such as TNF-α, NF-κB-p65, E-Cadherin and MMP-2 that are normally linked with the signaling of an inflammatory response in epithelial cells in cell-cell adhesion and apoptosis.

Tumor necrosis factor is an significant factor in cancer associated chronic inflammation and plays a vital role in tumor progression. In tumor microenvironment, the cytokine TNF-α triggers transcription factors and signaling pathways that are responsible for invasion and metastasis of malignant cells [36]. It has been reported that, cancer cells are more dependent on transcription factors and they could regulate the expression of transcription factor Snail to induce Epithelial-Mesenchymal Transition (EMT) and thereby stimulate tumor cell metastasis [37]. Inhibition of cell survival signals such as NF-kB by combined therapy is essential, since TNF-α promotes the continued existence of the tumor cells by the stimulation of genes encoding NF-κB dependent anti-apoptotic proteins. Abundant level of TNF-α are produced by tumor cells and enable survival of the tumor cells in an NF-κB dependent fashion. Tumor promoting cascade is initiated through MMPs which increased tumor migration and metastasis [38].

NF-κB, a significant transcription factor, which is involved in the regulation of gene expression and apoptosis [39,40]. NF-κB activates downstream pathways involving TNF-α, Bcl-2 and STAT-3 and thereby inhibits apoptosis [39,41]. NF-κB expression has been found to be upregulated only in NSCLC tissue than SCLC and is related with aggressive cancer cell metastasis among NSCLC patients [42]. Therefore, down regulation of the expression of NF-κB could be an efficient treatment approach for NSCLC patients [43,44]. In this study, we have demonstrated the dose dependent decrease in the inflammatory mediators expression, TNF-α and NF-κB, in A459 cell line by pyrazole. Pyrazole downregulated the TNF-α mRNA and protein expression at definite time points in A549 cells which is probably one of the inflammatory and signaling pathway mediators that trigger EMT and promotes tumor metastasis. The inhibition of NF-κB expression particularly inhibits TNF-α induced cell proliferation of human NSCLC A549 cells. Therefore, inhibition of TNF-α and NF-κB could be an effective and a promising strategy to combat NSCLC.

E-cadherin has a well-defined role in the production and maintenance of adherent junctions in epithelial cell-cell communication [45]. The loss of its function or impaired expression enables the cancer cells to separate from the constraints of the surrounding cells that allow them to invade to adjacent tissues

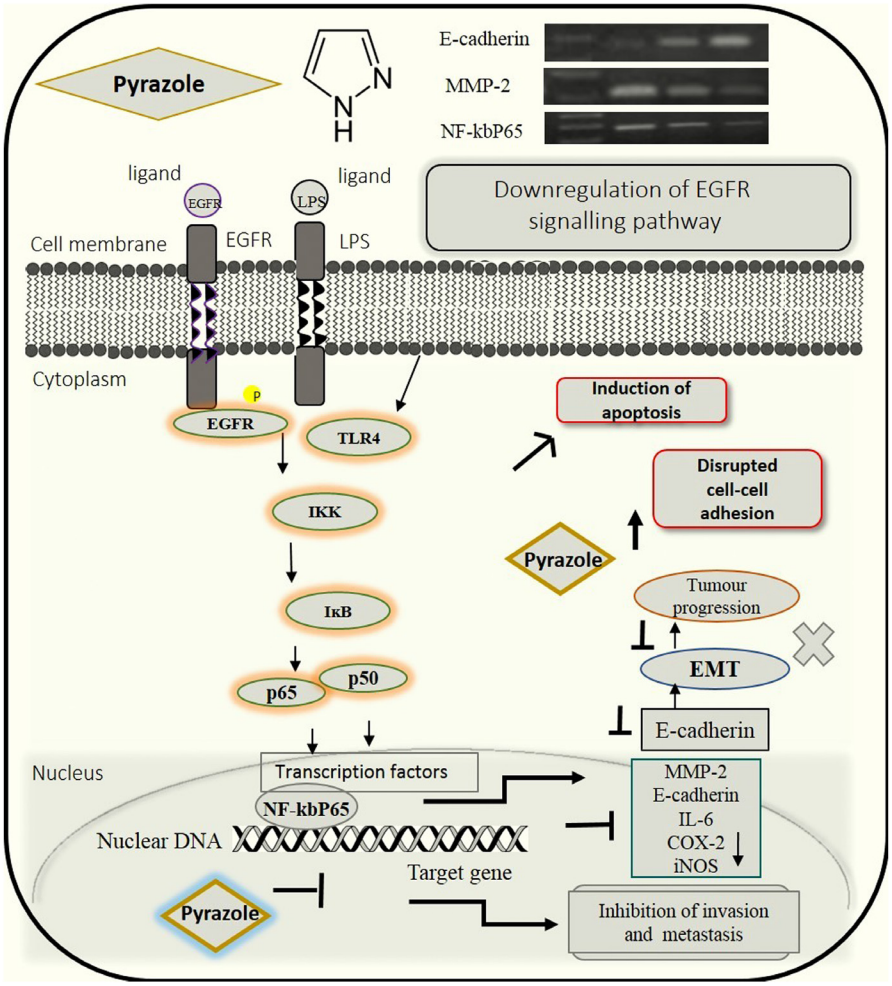

Fig. 8. Overall, pyrazole significantly up-regulate E-cadherin expression and regulated MMP-2 expression in A549 cells. Moreover, pyrazole inhibited the expression of TNF-α and NF-κB-p65 which specifically blocked NF-κB activation which was stimulated by TNF-α. The above anti-cancer effect was also substantiated by the role of pyrazole in regulating LPS-induced levels of cytokines and inflammatory mediators such as IL-6, TNF-α, iNOS and COX-2 in A549 cells as represented in the Fig. 8.

[45]. Impaired expressions of E-cadherin have been reported among NSCLC patients and are often associated with lymph node metastasis [46]. Therefore, E-cadherin is potential target for signaling pathways that are responsible for proliferation, migration and apoptosis [47]. MMPs are greatly expressed in tumor cells and are mainly associated with tumor angiogenesis and metastasis that lead to death of cancer patients [48–50]. Inhibition of MMP expression has been an early target in preventing cancer metastasis [52,53]. The author reported that patients with NSCLC showed over expression of MMP-2 in lung tumor tissue and this was related with aggressive metastasis [54].

The current study further signifies the effects of pyrazole as an anti-cancer drug on the migratory NSCLC cell line A549. Transcription of different genes like adhesion molecules, which are well known to regulate signaling pathways, are inhibited by pyrazole, and E-cadherin and MMP-2 are considered to be effective targets of pyrazole [47]. The present study reveals that treatment with pyrazole significantly up-regulated the E-cadherin gene and protein expression, in a timely manner in A549 cells and also significantly down regulated the mRNA and protein expression of MMP-2. Restoring E-cadherin gene expression in the pyrazole treated A549 cells resulted in the probably prevention of acquisition of metastatic potential of these cells. Nawrocki Raby et al. (2003) [51] reported that E-cadherin could control the expression of MMPs in order to support the cell stability and promote the inhibition of lung tumor cell progression. The present data reveal the down regulation of MMP-2 at mRNA and protein levels of A549 cells on pyrazole treatment.

We also observed that pyrazole inhibits the invasion of A549 cells, and hence metastasis, by suppressing TNF-α and NF-κB-p65 activation and thereby restoring E-cadherin and down regulating MMP-2 in NSCLC cells. The observations reveal that pyrazole could target the TNF-α signaling pathway thus inhibiting nuclear translocation of NF-κB, specifically, thereby preventing A549 cell proliferation and metastasis. The study also reveals that pyrazole up-regulated E-cadherin expression and down regulated MMP-2 expression which ensured the maintenance of adherent junctions of the cells and could restrained the interaction with the surrounding cells, preventing the cancer cells to metastasize. EGFR mediated signaling pathway would be the important preferred therapeutic target for treatment of NSCLC. Our earlier study revealed that pyrazole significantly blocked the EGFR signaling pathway and Bax, Bcl-2, which probably has a role in caspase-3 activation [8]. Pyrazole down-regulated the expression of MMP-2 suggesting that, pyrazole inhibits the expression of EGFR tyrosine kinase signaling pathway that controls aberrant MMP-2 expression and prevents invasion and metastasis.

Overall, pyrazole significantly up-regulate E-cadherin expression and regulated MMP-2 expression of human NSCLC A549 cells. Also, pyrazole supressed the expression of TNF-α and NF-κB-p65 subunit which specifically inhibited the NF-κB activation which was initiated by TNF-α. The above anti-cancer property was also substantiated by the role of pyrazole in regulating LPS-induced levels of predominant inflammatory mediators (IL-6, TNF-α, iNOS and COX-2) and cytokines in lung cancer cells. The current study reports the mode of action behind the anti-cancer and anti-metastatic activity of pyrazole in A549 cells by blocking the NF-κB signaling pathway and restoring E-cadherin expression. These observations indicated that 1,2-diazole suppressed TNF-α mediated MMP-2 gene and protein expression by blocking NF-κB stimulation in A549 cells. The observations of the present study also open a possibility for the use of a naturally derived therapeutic compound as a possible remedial agent that targets inflammatory and other cell signaling pathway mediators for potential human NSCLC treatment.

CRediT authorship contribution statement

Venugopal Vinod Prabhu: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Perumal Elangovan:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology. **Sivasithambaram Niranjali Devaraj:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration. **Kunnathur Murugesan Sakthivel:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

No conflict of Interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2021.e00594.

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