The Toxicity of Cooking Oil Fumes on Human Bronchial Epithelial Cells Through ROS-Mediated MAPK, NF-κB Signaling Pathways and NLRP3 Inflammasome

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Research Article

Keywords: Cooking oil fumes, Beas-2B cells, NF-κB signaling pathway, MAPK signaling pathway, Oxidative stress, Inflammation.

DOI: https://doi.org/10.21203/rs.3.rs-604026/v1

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Abstract

Cooking oil fumes (COFs) are main pollutants in kitchen and indoor air, which threaten human health. Exposure to COFs can cause respiratory diseases and impair pulmonary function. To investigate the toxicity of COFs on human bronchial epithelial cells (Beas-2B) and explore the underlying mechanisms, MTT assay was conducted to detect the viability of Beas-2B. Intracellular reactive oxygen species (ROS) levels and nitric oxide (NO) levels were determined with DCFH-DA assay and DAF-FM assay. The expression of genes involved in inflammation were measured with quantitative real-time PCR (qRT-PCR). The phosphorylation and the expression of proteins related to Mitogen-activated protein kinase (MAPK), NF-κB signaling pathways were measured with western blot. Our results revealed that COFs decreased Beas-2B cells viability, increased the ROS levels and NO levels in cells and induced apoptosis in Beas-2B cells. The results of qRT-PCR and western blot showed that the expression of NLRP3, p65, iNOS, IL-1β, and the factors related to oxidative stress and inflammation increased, NF-κB signaling pathway and MAPK signaling pathway were activated. This study provided some useful information to alleviate the toxicity of COFs and revealed the possible mechanism for the damage on respiratory system induced by COFs.

Introduction

With the rapid development of urbanization, indoor activities play a crucial role in various social activities and indoor pollution comes into our vision gradually (Jonathan M. Samet 1993). Indoor air pollution, as a kind of indoor pollution, has been recognized as a major public health problem (B. Berglund 1992; Smith 2002). Previous research has shown that nearly 3 billion people around the world were exposed to poor indoor air quality caused by heating, lighting and the burning of coal for cooking (Wang et al. 2018). Approximately 4.3 million people died from the exposure of indoor air pollution every year (Gonzalez-Martin et al. 2021). The extent and scope of indoor air pollution in developing countries are much more critical than that in developed countries (Ellegard 1997). Cooking, as main human activity, is one of the most common sources of indoor air pollution and can release a lot of pollutants (Ellegard 1997; Sofuoglu et al. 2015; Wang et al. 2018).

COFs pollution produced by cooking usually includes ketones, aldehydes, hydrocarbons, fatty acids, alcohols, aromatic compounds, esters, heterocycles, and lactones is a serious health risk in china due to the cooking manners (Ding et al. 2020; Lee and Gany 2013). In China alone, the number of people exposed to COFs in 2018 has reached to 240 million and most of whom were middle-aged women (Liu et al. 2020). Women exposed to COFs are at higher risk for chronic bronchitis, for pregnant women, such exposure can also affect the fetus and increase the risks of adverse birth outcomes (Hou et al. 2018; Vavalà et al. 2014; Zhu et al. 2019). Studies have shown that exposure to COFs increased the risk of respiratory diseases, such as airway infection, chronic obstructive pulmonary disease, tuberculosis and asthma (Allen L. Robinson 2006; Sjaastad et al. 2010; Sjaastad and Svendsen 2009; Svendsen et al. 2002). In addition, cardiovascular disease, abnormal pregnancy and cervical cancer are also related to COFs (Anderson 1979; Kirk R Smith 2000). The occurrence of these diseases may be related to cytotoxic
damage caused by COFs, it has been reported that the use of ventilation equipment can reduce the risk of cardiopulmonary death to 40% within five years (Ma et al. 2021).

It has been reported that the major compound contained in COFs can affect ROS and induce oxidative DNA damage in human lung carcinoma cells (Wu and Yen 2004). The apoptotic effect of COFs in fetal lung type II-like epithelium cells was investigated, which revealed that COFs induced apoptotic via mitochondrial intrinsic apoptosis pathway (Che et al. 2014). Trans, trans-2,4-decadienal (tt-DDE), a composition of cooking oil fumes, could induce oxidative stress and endoplasmic reticulum stress in human corneal epithelial cells (Yan et al. 2020). But the toxicity of COFs on Beas-2B has rarely been reported.

The purpose of this study was to investigate the toxicity of COFs on Beas-2B cells, explore the signaling pathways involved inflammation and oxidative stress and provide some experimental basis for studying the cytotoxic mechanism of COFs on respiratory system.

**Materials And Methods**

**Cooking oil fumes**

COFs samples were collected by referring to Ding et al (Ding et al. 2020). Briefly, The COFs was produced from heating peanut oil (200 mL) by an electric heater in iron pan and the temperature was kept at the smoke point (280 ± 10 °C). The fumes were collected into a brown bottle for preservation and stored at -80 °C. Different concentrations of COFs were dissolved in dimethyl sulfoxide (DMSO) when used.

**Cells and culture conditions**

Beas-2B cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured in high glucose DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 100 U/mL penicillin (C0222, Beyotime, Shanghai, China), 10% fetal bovine serum (FBS, Gibco, New York, NY, USA) and 100 μg/mL streptomycin (C0222, Beyotime, Shanghai, China) at 37 °C in 5 % CO₂.

**Cell viability assay**

Beas-2B cells were seeded in 12-well plates at density of 3×10⁵ cells/well, treated with COFs at concentration of 200 μg/mL for 12 h, 24 h and 48 h and observed with electron microscope (Olympus CX22LED microscope 10×magnification, Olympus Corporation, Tokyo, Japan) (Liu et al. 2018). The cells viability was detected with MTT assay (Cao et al. 2021). Briefly, Beas-2B cells were seeded at the density of 1×10⁴ cells per well in 96-well plate, then the cells were cultured in a humidified atmosphere of 5 % CO₂ at 37 °C (Lab-Line CO₂ Incubator, Merlose Park, USA) and treated with COFs (100, 150, 200, 250, 300, 350, 400, 600, 800 μg/mL) for 24 h. Thiazolyl blue tetrazolium bromide (MTT) was added to each well. The absorption at 490 nm was measured with microplate reader (Varioskan® Flash, Thermo Fisher Scientific...
Inc.), the survival rate was calculated with OD_{experimental group}/OD_{control group}×100% (Park and Park 2009).

**Apoptosis assay**

After 24 h exposure to COFs (200, 400, 600 μg/mL), Beas-2B cells were digested with trypsin without EDTA, washed with 1 mL PBS twice and centrifuged 5 min at 1000 rpm. According to the manufacturer’s protocols, apoptosis was measured with double stained with Annexin-V-FITC apoptosis detection kit and propidium (PI) (eBioscience, CA, USA). Briefly, 100 μL binding buffer, 1 μL Annexin V-FITC, and 1 μL PI staining solution were added to Beas-2B cells in 6-well plates, the cells incubated at room temperature for 5 min and the apoptosis was detected with a flow cytometry (BD, FACS Cantoll) (Wang et al. 2019).

**mRNA expression measurement**

Total cellular RNA was extracted from Beas-2B cells with RNA isolation kit (Sigma-Aldrich) according to the manufacturer’s instructions. The mRNA was transcribed into cDNA with cDNA synthesis kit. Then, qRT-PCR was conducted by using the SYBR Premix Ex Taq (Tli RNaseH Plus) with Applied Biosystems 7500 Real-Time PCR system (Appbice Applied Biosystems Trading Co., Ltd., Shanghai, China). The PCR thermocycling conditions were as follows: denaturation at 95 °C for 40 seconds followed by 40 cycles of 95 °C for 10 seconds and 60 °C for 35 seconds (Zhong et al. 2019). The primer pairs for human IL-6, iNOS, ICAM-1, IL-1β, IL-8, COX-2, NLRP3, CRP, TNF-α, IL-17, IL-18 and β-actin were shown in table 1.

**Table 1 Sequences of primers and probes used in the present study**

| Gene     | Primer Forward [5→3] | Primer Reverse [5→3] | Product |
|----------|----------------------|----------------------|---------|
| TNF-α    | AGGACGAACATCCAACCTTCCCAA | TTTGAGCCAGAAGAGGTGAGGT | 92      |
| iNOS     | TGCAGACACGTGGTACTCC   | GGTAGCCAGCATAGCGGATG  | 130     |
| COX-2    | TTCAAATGAGATTGTGGAAAAATTGT | AGATCATCTCTGCGTAGTGATCT    | 146     |
| IL-1β    | TCCAGGGACAGGATGGAG    | TCTTTCACACAGCAGGACAG    | 133     |
| IL-6     | ATGAACTCCTTCTCACAAGGC | GAAGAGCCCTCAGGCTGGACTG  | 166     |
| IL-8     | AGCTCTCTTGGAAGGTGCA   | AATTTCTGTGTGGCGCAGT     | 148     |
| ICAM-1   | AGC TTC TCC TGC TCT GCA AC | GTC TGC TGG GAA TTT TCT GG | 146     |
| NLRP3    | CTTCTTTCCAGGTTTGCTGC  | TCTCGCAGTCACAATTCTTCT    | 212     |
| CRP      | GGGCCCTTCCAGTCTACTGTC | TCTCGCAGTCACTTCATATTCT | 156     |
| β-Actin  | ACCCGCCGAGACGCCGTCGCCGCCC | TGGTGCCAGGCCCCTTCACA   | 133     |
| IL-17    | TGGGAAGACCTGATTTGTGT | GGATTTCGTGGGATTGTGAT   | 84      |
| IL-18    | AGTCAGCAAGGAATTGTCTCC | GAAGCAGCTGTGGGAAGTCTG   | 135     |
Measurement of intracellular ROS

The levels of ROS were measured with the fluorescent probe 2’, 7’-dichlorofluorescein diacetate (DCFH-DA) (KeyGen Biotech, Jiangsu, China). In short, Beas-2B cells were treated with COFs (200, 400, 600, 800 μg/mL) for 24 h, then washed with PBS once and probed with 10 mM DCFH-DA for 30 min at 37 °C. The positive fluorescent signals were imaged under inverted fluorescence microscope (Park et al. 2008).

Measurement of intracellular NO

Intracellular NO was measured with the NO-specific cell-penetrable fluorescent probe 3-Amino, 4-aminomethyl-2, 7-difluorescein, diacetate (DAF-FM) (KeyGen Biotech, Jiangsu, China). Briefly, Beas-2B cells were seeded in 12-well plates at a density of 1 × 10^6 cells/well and treated with different concentrations COFs (200, 400, 600, 800 μg/mL) for 24 h. After removal supernatant, the cells was washed once and probed with 10 mM DAF-FM for 35 min at 37 °C. The positive fluorescent signals were observed under inverted fluorescence microscope (× 10) (Li et al. 2020).

Cell signaling pathway analysis

Western blot analysis was carried out according to our previous study (Cao et al. 2020b). After treatment with COFs (0, 200, 400, 600 μg/mL) for 24 h, Beas-2B cells were harvested and lysed, total protein extracts and nuclear extracts were prepared with total protein and nuclear protein extraction kits. Then the protein concentration was measured by BCA method. Protein samples were separated by 10% SDS-PAGE gels and transferred to PVDF membranes according to standard electroblotting procedures. After blocked with 5% non-fat milk for 1 h, the membrane was incubated with anti-Bax, anti-Bcl-2, anti-cleaved caspase-1, anti-cleaved caspase-3, anti-IkB, anti-p-IkB, anti-p65, anti-PCNA, anti-β-actin, anti-JNK, anti-ERK, anti-p38, anti-p-JNK, anti-p-ERK, anti-p-p38 monoclonal antibodies (at 1:2000 dilutions), then incubated with secondary antibodies. Finally, bands were observed by using an enhanced chemiluminescence kit. The relative level of total protein was normalized to β-actin and the relative level of nuclear protein was normalized to PCNA (Cao et al. 2020a; van der Stel et al. 2020).

Statistical analysis

The data were presented as means ± standard deviation (SD) at least three independently performed experiments. The date analyses were performed with one-way analysis of variance (ANOVA) with SPSS 21.0 (IBM SPSS, Armonk, NY, USA). P < 0.05 and P < 0.01 revealed statistical significance.

Results

Cytotoxicity of COFs on Beas-2B cells

Morphological changes of Beas-2B cells were observed under the phase contrast microscope. After COFs (200 μg/mL) treatment, the shape of cells changed from tiled to round, and even floating, indicating that the decreased viability of Beas-2B cells was mainly attributable to cells death (Fig. 1A).
The toxicity of COFs (100, 150, 200, 250, 300, 350, 400, 600, 800 μg/mL) on Beas-2B cells was evaluated by MTT assay. As shown in Fig. 1B, with the increasing concentration of COFs, the cell viability decreased significantly and showed a dose-manner (p < 0.01). Moreover, under the treatment of COFs at the concentration of 600 μg/mL of COFs, approximately 50% decrease of cell viability was observed after 24 h in comparison with the group of control group, and 200, 400, 600 μg/mL COFs were selected for the subsequent experiments.

**Oxidative stress induced by COFs in Beas-2B cells**

The accumulation of ROS can reflect the degree of oxidative stress and play a key role in oxidative damage (Deanfield et al. 2007; Li et al. 2020). The effects of COFs on ROS production were detected with DCFH-DA fluorescence probe. As shown in Fig. 2, after the cells were treated with different concentrations of COFs (200, 400, 600 μg/mL), with the increase of COFs concentration, the fluorescence intensity was 1.54, 2.04 and 2.51 times compared with the control group respectively. These results revealed that ROS levels in the cells increased in a dose-dependent manner (p < 0.01).

**Inflammation induced by COFs in Beas-2B cells**

The NO concentration can be regarded as an indicator of inflammatory reaction (Giuseppe Cirino 2006). As shown in Fig. 3, the fluorescence intensity in COFs (200, 400, 600 μg/mL) treatment groups were 1.21, 1.33 and 2.03 times compared to the control group respectively. With the increase of COFs concentration, NO levels of COFs treatment groups increased significantly (p < 0.01), which suggested that the inhalation of COFs might lead to inflammation.

**Apoptosis induced by COFs in Beas-2B cells**

The apoptosis ratio was detected with flow-cytometry assay (Dhib et al. 2017). As shown in Fig. 4, with the increase of COFs concentration (200, 400, 600 μg/mL), apoptosis ratios were 10.85 ± 1.1%, 19.7 ± 1.3%, and 24.6 ± 1.8% respectively, which were significantly higher than that in the control group (5.6 ± 1.4%). Moreover, with the increasing concentrations of COFs, the percentages of apoptosis cells increased significantly and showed a dose dependent manner. These results indicated that Beas-2B cells proliferation was inhibited and apoptosis was activated after the treatment of COFs.

**mRNA expression induced by COFs in Beas-2B cells**

After the treatment of COFs at 200 μg/mL, the levels of NLRP3, p65, iNOS, TNF-α, COX2, CRP, ICAM-1, IL-1β, IL-6, IL-17, IL-8 and IL-18 in Beas-2B cells were measured with quantitative real-time PCR (qRT-PCR) (Cao et al. 2019). As shown in Fig. 5, the mRNA expression levels of those genes increased after the treatment of COFs at 200 μg/mL compared with the control group (p < 0.01).

It is known that iNOS plays an important role in inflammation and infection (Maggie Bargouti 2009). The result showed that the expression level of iNOS increased significantly (p < 0.01) after 200 μg/mL COFs
treatment. In addition, the expression of NLRP3, IL-1β, IL-17 and other inflammatory factors were also increased ($p < 0.01$) (Fig. 5) (Pinkerton et al. 2017; William Y. Park and David R. Park 2001).

**Apoptosis-related proteins expression induced by COFs in Beas-2B cells**

To further investigate the possible mechanism of apoptosis, the expression of Bcl-2-associated X (Bax), B-cell lymphoma-2 (Bcl-2), cleaved caspase-3 and cleaved caspase-1 were detected (Wang et al. 2017). Compared with the control group, with the increase of COFs (200, 400, 600 $\mu$g/mL), the ratio of Bax/Bcl-2 increased significantly ($p < 0.01$) (Fig. 6). When the concentration of COFs was 400 $\mu$g/mL and more, the levels of cleaved caspase-1 and cleaved caspase-3 proteins were increased compared with the control group significantly ($p < 0.01$). Furthermore, the level of cleaved caspase-3 in 600 $\mu$g/mL COFs-treated group was 2.51 times compared with the control group ($p < 0.01$). These results showed that COFs increased the ratio of Bax/Bcl-2, the expression of cleaved caspase-3 and cleaved caspase-1 and induced apoptosis in Beas-2B cells.

**Effect of COFs on the MAPK signaling pathway**

In order to further study the oxidative stress pathway mediated by COFs, the phosphorylation of extracellular signal-regulated kinase extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK were studied. As shown in Figure. 7A, with the increasing concentration of COFs (200, 400, 600 $\mu$g/mL), the ratio of p-ERK/ERK showed significant increase in all the concentrations, the ratio of p-JNK/JNK showed significant difference in the concentration of 600 $\mu$g/mL ($p < 0.01$), while p-p38/p38 significantly increased in the groups of 400 $\mu$g/mL and 600 $\mu$g/mL compared with the control group respectively (Figure. 7B).

**Effect of COFs on the NF-κB signaling pathway**

The expressions of p65 and IκB in COFs (200, 400, 600 $\mu$g/mL) groups were measured to assess the contribution of the NF-κB pathway to the inflammation effect on Beas-2B. 200, 400, 600 $\mu$g/mL COFs upregulated the rate of p-IκB/IκB significantly ($p < 0.01$) (Fig. 8). Furthermore, it could be seen that with the increase of COFs concentration, the expression of p65 in nucleus increased ($p < 0.01$) and the change of total p65 was not significant, indicating that NF-κB signaling pathway was activated.

**Discussion**

Lung is vulnerable and sensitive to the exposure to air pollutants. According to a Norwegian survey, employees in restaurant exposed to oil fumes were more likely to die from respiratory diseases such as asthma and emphysema (Svendsen et al. 2002). Exposure to COFs increased the probability of acute respiratory infection in children according to a study in Thailand (Juntarawijit and Juntarawijit 2019). A longitudinal study in Chinese military cooks revealed that exposure to COFs caused oxidative DNA damage and lipid peroxidation (Lai et al. 2013). Lungs of mice after inhalation of COFs exhibited diffuse cellulose exudation from alveolar and bronchus, severe hemorrhage and moderate interstitial
lymphoplasmacytic infiltration (Wu et al. 2004). However, the cytotoxicity induced by COFs in Beas-2B cells has rarely been reported. In this study, the effect of COFs on oxidative stress, inflammation and apoptosis was explored, and the preliminary mechanism of COFs toxicity on Beas-2B cells was investigated.

Oxidative stress is a common manifestation of cell damage (Lee et al. 2013). ROS produced by environmental factors can cause nuclear and mitochondrial damage, cell metabolism dysfunction, inflammation and apoptosis (Aschauer et al. 2015; D. L. Carlisle 2000; Son et al. 2010). After COFs treatment, the accumulation of ROS in Beas-2B cells was significantly increased, which was similar to the damage of deoxynivalenol on HT-29 cells (Guo et al. 2021a). The MAPK signaling pathway is closely related to oxidative stress and promotes the process of cell apoptosis, containing the ERK, JNK and p38 MAPK (Ebegboni et al. 2019). ROS accumulation can active MAPK pathway and induce oxidative stress (Cao et al. 2021). The phosphorylation of ERK, JNK and p38 was upregulated significantly after COFs treatment, suggesting that the MAPK signaling pathway in BEAS-2B cells were activated, which was in consistence with the mechanism about the toxicity of copper in mice (Guo et al. 2021b). Therefore, the mechanism of oxidative stress induced by COFs may be the activation of MAPK signaling pathways.

Inflammation is the primary factor to evaluate the effect of particles in the air on human health (Yao et al. 2021). NF-κB (p65), as a downstream target of p38 MAPK, plays a key role in cell proliferation, apoptosis and inflammatory (Wang et al. 2020). Inactive p65 usually remains in the cytoplasm and activated p65 is usually transported to the nucleus to perform functions (Gholinejad et al. 2019). Expression of iNOS is closely related with the upregulation of p65 and can produce NO which aggravates the inflammatory in cells (Nathan and Ding 2010; Park et al. 2005). COFs treatment upregulated the mRNA expression of iNOS, increased the concentration of NO and induced inflammatory in BEAS-2B cells and this result was same as the mechanism of Toll-like receptor 7 (TLR-7) in B-cell chronic lymphocytic leukemia (B-CLL) cells (Hammadi et al. 2008). C-reactive protein (CRP) is an acute phase protein and plays an important role in inflammation and infection when the body is stimulated by negative effects such as microbial invasion or tissue damage (Schlereth et al. 2014). Our results showed that the concentration of NO increased, inflammatory reaction occurred and inflammatory factor IL-1β, IL-6, IL-8, IL-17, IL-18, TNF-α and CRP were upregulated after COFs treatment, and this result was consistent with the inflammation induce by PM 2.5 (Geng et al. 2019). NLRP3 is the most typical and widespread inflammation in pulmonary inflammatory diseases (Cao et al. 2020b). The activation of NLRP3 inflammatory complex is important to fight against respiratory tract infection, however, over activation may lead to serious diseases (Pinkerton et al. 2017). p65 is an important transcription factor regulating cell growth, proliferation, apoptosis and carcinogenesis (Liu et al. 2018). It has been reported that cigarette smoke could increase the NLRP3 inflammatory complex and activate NF-κB pathway in THP-1 cells (Mehta and Dhawan 2020). In this study, the distribution of p65 in the nucleus increased and the phosphorylation of IκB increased after COFs treatment, and this result was consistent with the toxicity induce by acrylamide in HepG2 cells (Bo et al. 2020). the mechanism of COFs induced inflammatory response of Beas-2B cells may be to activate NF-κB signaling pathway.
Apoptosis is programmed cell death and plays an important role in maintaining cell homeostasis (Ebegboni et al. 2019). Apoptosis is also regulated by many genes, the Bcl-2 is a potent inhibitor of apoptosis and plays a regulatory role in the process of cell apoptosis (Krishnaswamy Kannan 2000). The proportion of pro-apoptotic members to the level of Bax in mitochondria is closely related to cell survival or apoptosis (Ingo Schmitz 2000). The ratio of Bcl-2 to Bax determines the cell’s life and death, Bcl-2 family proteins transfer from the cytoplasm to the mitochondrial membrane and mediate the activation of caspase, mediating the mitochondrial endogenous apoptosis pathway (Cao et al. 2021; Yang et al. 2019). It has been reported that crotonaldehyde could induce Beas-2B cells apoptosis by regulating the expression of caspase family proteins (Wang et al. 2019). In this experiment, COFs could significantly upregulate the expression of Bax, cleaved caspase-1, cleaved caspase-3, decrease the expression of Bcl-2, the same mechanism was also addressed in the apoptosis caused by chlorogenic acid in chondrocyte cells (Kulyar et al. 2021). COFs could induce apoptosis by regulating the mitochondrial apoptosis pathway.

Conclusion

COFs exposure led to the decrease of cell viability, the accumulation of ROS and NO, oxidative stress, inflammation and apoptosis. The abnormal expression of apoptosis-related proteins Bax, Bcl-2, cleaved caspase-1 and cleaved caspase-3 in BEAS-2B cells revealed that mitochondria-mediated intrinsic apoptosis pathway was activated. COFs increased the phosphorylation of related proteins in MAPK family JNK, ERK and p38 and led to oxidative stress (Fig. 9). COFs exposure increased the phosphorylation of IκB and promoted p65 translocate into nuclear and activated NF-κB signaling pathway (Fig. 9). This study gave a new understanding of the toxicity of COFs on BEAS-2B cells and provided scientific data to assess its potential risks on lung disease induced by COFs.

Declarations

Authors’ contributions Conceptualization, Xiangyu Cao and Jianli Liu; writing – original draft preparation, Mingyang Fu and Jingyi Miao; investigation, Yueling Sun and Rugang Zhu; statistical analysis, Chengying Liu and Ruochen Bi; writing – review and editing, Shuai Wang and Jianli Liu; funding acquisition, Xiangyu Cao and Jianli Liu. All authors have read and agreed to the published version of the manuscript.

Funding This study was supported by the Project Supported by Scientific Research Fund of Liaoning Provincial Education Department (No.LFW201903; No. LQN201714), Project Supported for Youth and Middle-aged Science and Technology Innovative Talents of Shenyang City (No.RC200137), Natural Science Foundation of Liaoning Province (No.2019-ZD-0198).

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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Figures

**Figure 2**
Effect of COFs on ROS levels of Beas-2B cells. Data were expressed as mean ± SD of three independent experiments. **p < 0.01 compared with the control group.

Figure 3

Effect of COFs on the levels of NO in Beas-2B cells. Data were expressed as mean ± SD of three independent experiments. **p < 0.01 compared with the control group.

Figure 6
COFs regulated the expression of apoptosis-associated proteins Bax, Bcl-2, cleaved caspase-1 and cleaved caspase-3. Data were expressed as mean ± SD of three independent experiments. **p < 0.01 compared with the control group.

Figure 7

Effects of COFs on MAPK signaling pathway in Beas-2B cells. (A). Western blot analysis of protein expression of ERK, JNK and p38 protein. (B). COFs increased the protein expression of phosphorylation ERK, JNK and p38. Data were expressed as mean ± SD of three independent experiments. **p < 0.01 compared with the control group.

Figure 8
COFs regulated the expression of inflammatory pathways proteins p65, IkB and p-IκB. Data were expressed as mean ± SD of three independent experiments. **p < 0.01 compared with the control group.

**Figure 9**

COFs induced BEAS-2B cells damage through oxidative stress, inflammation and activated mitochondria-mediated intrinsic apoptosis pathway.