REPUBLIC OF TURKEY
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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

INVESTIGATION OF THE EFFECT OF ELLAGIC ACID ON THE EXPRESSION OF SOME APOPTOTIC PROTEINS IN LUNG INJURY-INDUCED RATS

Yousif Taha HUSSEIN

Master Thesis
DEPARTMENT OF BIOLOGY

Molecular Biology Program
JANUARY – 2020

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This thesis was prepared according to the rules of Firat University, Graduate School of Natural and Applied Sciences. It was evaluated by the jury members who have signed the following signatures and accepted unanimously as a result of the defense made available to the academic listeners.

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This thesis was registered at the meeting of the Board of Directors of the Institute on 11/ 12/ 2019

Signature

Prof. Dr. Soner ÖZGEN

Director of the Institute

DECLARATION

This thesis “Investigation of the effect of ellagic acid on the expression of some apoptotic proteins in lung injury-induced rats” was writing according to the thesis writing rules of the Firat University, Graduate School of Natural and applied Sciences. All the information in my thesis is correct, scientific ethics in producing and presenting information that I have written in accordance with the rules, I have cited all the sources to receive the title of the data and the information that I have provided here. I have never used the data and informations that provided here in order to get a degree in anywhere.

06/ 01/ 2020

Yousif Taha HUSSEIN

PREFACE

In this thesis, the effect of ellagic acid on the expression of some apoptotic proteins in lung injury-induced rats was investigated. The results presented in this thesis are promising and may contribute to the literature.

Praise be to Allah, lord of the worlds, the entirely merciful, the especially merciful.

I would like to express my deepest gratitude to my supervisor, Assoc. Professor Dr. Abdullah ASLAN for providing me with an opportunity to work on this research. His guidance, support and constant help have made this thesis/dissertation possible. His guidance throughout the process without losing faith in me has been most inspiring and educational.

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ABSTRACT

Investigation of the Effect of Ellagic Acid on the Expression of Some Apoptotic Proteins in Lung Injury- Induced Rats

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Master Thesis

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Phytochemicals is considered as one of the most effective and safe alternative therapy against oxidative-linked lung diseases. Ellagic acid (EA), a natural polyphenolic component of fruits, nuts and vegetables, are partly responsible for their therapeutic health effects against oxidation-related diseases. In this study, we investigated the ameliorative effect of EA on lung damage induced by Carbon tetrachloride (CCl_4) in Wistar albino rats. Thirty-six male rats (n=36, 8 weeks old) were divided into 4 groups, each with 9 rats. The groups were: Control Group: served as control received standard diet; EA Group and CCl_4 Group were administered EA (10 mg/kg body weight (b.w.), intraperitoneal (ip)) and CCl_4 (1.5 ml/kg b.w. ip.), respectively; CCl_4 + EA Group administered EA plus CCl_4 as in the EA Group and the CCl_4 Group. The rats were decapitated at the end of experimental period of 8 weeks and the lung tissue were examined. CCl_4 induced rats showed elevation in the expressions of inflammatory proteins, Nuclear factor kappa b (NF-kB), Cyclooxygenase-2 (COX-2) and Tumor necrosis factor alpha (TNF-α); and the indicator of lipid peroxidation, Malondialdehyde (MDA). Intraperitoneal administration of EA significantly reduced the levels of these markers. EA administration increased the protein expression levels of nuclear factor erythroid 2 related factor 2 (Nrf-2) and enhanced the activity of glutathione (GSH) and Catalase enzyme (CAT). In addition, EA administration increased the expression levels of caspase-3, and decreased B-cell lymphoma-2 (Bcl-2). In conclusion, these results establishes the protective role of EA in the treatment of lung damage and that in the future this may have the potential to be used as a medication for the prevention or attenuation of lung diseases.

Keywords: Ellagic acid, lung damage, TNF-α, COX-2, caspase-3, Nrf-2.

ÖZET

Ellagik Asitin Akciğer Hasarı Oluşturulmuş Sıçanlarda Bazı Apoptotik Proteinlerin İfadesine Etkisinin Araştırılması

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Fitokimyasallar oksidatif hasara bağlı akciğer hastalıklarına karşı en etkili ve güvenli alternatif tedavilerden biri olarak kabul edilmektedir. Meyveler, fındıklar ve sebzelerin doğal polifenolik bir bileşeni olan ellagik asit (EA), oksidasyonla ilgili hastalıklara karşı terapötik sağlık etkilerinden kısmen sorumludur. Bu çalışmada, Wistar albino sıçanlarda Karbon tetraklorürün (CCl4) neden olduğu akciğer hasarı üzerinde EA'in koruyucu ve iyileştirici etkisi araştırılmıştır. 36 adet Wistar albino (n=36, 8 haftalık) sıçan kullanılmıştır. Sıçanlar 4 gruba ayrılmış ve her grupta 9 sıçan yer almıştır. Gruplar: (i) Kontrol Grubu: Standart diyet; (ii) EA Grubu: Standart diyet + EA verilen grup; (iii) CCl4 Grubu: Standart diyet + CCl4 verilen grup; (iv) CCl4 + EA Grubu: Standart diyet + CCl4 + EA verilen grup. Hayvanlar 8 hafta sonra dekapite edilerek akciğer dokuları incelenmiştir. Akciğer dokusunda kaspaz-3, bcl-2, Nrf-2, NF-kB, COX-2 ve TNF-α ekspresyon düzeyleri western blotlama tekniğiyle, lipit peroksidasyonu MDA (malondialdehit) analizleri, GSH ve katalaz düzeyi spektrofotometre ile belirlenmiştir. CCl4 ile indüklenen sıçanlarda, enflamatuar proteinlerin ifadelerinden; Nükleer faktör kappa-b (NF-kB), Siklooksijenaz-2 (COX-2) ve Tümör nekroz faktörü alfa (TNF-α) ve lipid peroksidasyon göstergesi Malondialdehit (MDA) değerlendirilmiştir. EA'in intraperitoneal uygulaması ile bu belirteçlerin seviyelerinin önemli ölçüde azaldığı görülmüştür. EA uygulaması, nükleer faktör eritroid 2 ile ilişkili faktör 2'nin (Nrf-2) protein ekspresyon seviyelerini, glutatyon (GSH) ve katalaz enziminin (CAT) aktivitesini arttırmıştır. Buna ilave olarak, EA uygulaması kaspaz-3 ekspresyon seviyelerini arttırmış ve B hücreli lenfoma-2'yi (Bcl-2) azaltmıştır. Sonuç olarak, EA'in akciğer hasarının tedavisinde koruyucu bir rolünün olduğu ve gelecekte akciğer hastalıklarının önlenmesi veya zayıflatılmasında bir ilaç olarak kullanılma potansiyeline sahip olabileceği kanısına varılmıştır.

Anahtar kelimeler: Ellagik asit, akciğer hasarı, TNF-α, COX-2, kaspaz-3, Nrf-2.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations

mg:	Milligram
kg:	Kilogram
ml:	Milliliter
µg:	Microgram
µl:	Microliter
ºC:	Centigrade
%:	Percent
b.w.:	Body weight
i.p.:	Intraperitoneal
EA:	Ellagic acid
ETs:	Ellagitannins
ROS:	Reactive oxygen species
DMSO:	Dimethyl sulfoxide
EDTA:	Ethylenediaminetetra acetic acid
H2O2:	Hydrogen peroxide
NADPH:	Nicotinamide adenine dinucleotide phosphate
PBS:	Phosphate Buffered Saline
PMSF:	Phenylmethylsulfonyl fluoride
RPM:	Revolutions per minute
SPSS:	Statistical Package for the Social Sciences
CCl_4:	Carbon tetrachloride
CCl3•:	Trichloromethyl radical
CCl3OO•:	Trichloromethylperoxyl radical
•OH:	Hydroxyl radicals

Abbreviations

NF-kB:	Nuclear factor kappa b
TNF-α:	Tumor necrosis factor alpha
COX-2:	Cyclooxygenase-2
Bcl-2:	B-cell lymphoma-2
Nrf-2:	Nuclear factor erythroid related factor 2
CAT:	Catalase enzyme
GSH:	Reduced glutathione
GSSG:	Oxidized glutathione
ALI:	Acute lung injury
COPD:	Chronic obstructive pulmonary disease
CS:	Cigarette smoke
ARDS:	Acute respiratory distress syndrome
ARE:	Antioxidant response element-bearing genes
O2•-:	Superoxide anion

1. INTRODUCTION

Oxidative stress is a key part of the chain of events that leads to lung injury and consequently to several disease conditions [1, 2]. There is a body of evidence implicating oxidative stress in the pathogenesis of numerous lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), Acute Lung Injury (ALI), pulmonary fibrosis and lung cancer [2-4]. Among these oxidation-linked lung diseases, lung cancer is the most frequently diagnosed cancer and ranks first cause of death from cancer (For both sexes combined) and COPD that predicts to become the world's third leading cause of death by 2030 [5, 6].

The lung, as the main respiratory organ, is an important target of oxidative damage from inhaled xenobiotic and toxic processes mediated by reactive oxygen species (ROS). Due to greater than 50 m2 surface area, it continuously exposed to endogenous and exogenous stimuli including environmental pollutant, medicinal agents, cigarette smoke (CS) and pathogens. When these exogenous compounds enter the body, they are degraded or metabolized and free radicals are produced as by-products. As such, the lung cells is relatively hyperoxic state compared with cells of other organs [1, 4, 7-9]. When prooxidants increase or antioxidant defense fails, oxidative stress causes severe molecular damage and tissue injury [10]. Oxidative stress may disturb the balance between gene expression of proinflammatory mediators and antioxidant responses in favor of proinflammatory mediators in the lungs [11]. The lung induces range of responses to protect itself from further oxidative-injury. Unfortunately, these responses frequently generate more damage and are associated with various oxidative-lung diseases [4].

The enhanced generation of free radicals and oxidative stress situation can be induced by a variety of exogenous chemicals. Among these exogenous chemicals, Carbon tetrachloride (CCl_4), a hepatotoxic compound, which is experimentally used for free radical generation in various organs including lungs [12-14]. The administration of CCl_4 induce transient tissue disorders due to generation of free radicals. CCl_4 is metabolized in the endoplasmic reticulum by enzymatic reduction of cytochrome P-450 to trichloromethyl radical (CCl3•) and rapidly form a highly reactive trichloromethyl peroxyl radical (CCl3OO•) in the presence of oxygen. The reactive free radicals formed by carbon tetrachloride can induces an adverse reaction and causes damage to lipids, proteins and nucleic acids [15, 16].

Antioxidants protect the lung from diseases resulting from oxidative damage by countering the reactive free radicals; therefore, antioxidant therapy can prevent diseases linked to free radicals [16]. It has been documented that good health can be sustained through the use of plants with high antioxidant content [17, 18]. Polyphenolic plant compound such as Ellagic acid (EA), an important component of fruits, nuts and vegetables, they are partially responsible for their

useful health impacts in several oxidation-related health conditions. Studies have shown the anti-inflammatory activity of EA and its derivative's in the airways and lungs [19, 20]. It has been also acknowledged for its anti-mutagenic, anti-clastogenic, anti-carcinogenic and antioxidant activities [21, 22]. These effects due to its inhibitory effect on Tumor necrosis factor alpha (TNF-α), Cyclooxygenase-2 (COX-2), Nuclear factor kappa b (NF-kB); and apoptosis-inducing activity via up-regulation of caspase-3 and down-regulation of B-cell lymphoma-2 (Bcl-2). In addition, studies showed that EA decreased lipid peroxidation marker, Malondialdehyde (MDA) in the cells and activate cellular rescue pathways through up-regulation of nuclear factor erythroid related factor 2 (Nrf-2) and activation of antioxidants and anti-oxidant enzymes [18, 23-33].

In the present study, we investigated the protective effect of Ellagic acid on CCl_4-induced lung damage in Wistar albino rats. For this purpose, we measured different protective role of EA such as inhibitory effect on some inflammatory mediators (e.g. NF-kB, TNF-α, COX-2) and regulatory effects on apoptotic proteins such as caspase-3 and Bcl-2. In addition, its effect on Nrf-2, catalase enzyme (CAT), Glutathione and Lung tissue MDA levels were also measured.

1.1. The Histological and Physiological Features of Lung

The lungs are a paired spongy-like, air-filled organ located on either side of the thoracic cavity. The inhaled air enters the lungs from the trachea via its right and left tubular branches, named the bronchi and ultimately reaches the alveoli wherein the gas exchange occurs (Figure 1.1) [34]. The histological characteristics of the lungs are the histological properties of its components, bronchi, bronchioles and alveoli. The bronchi are lined by pseudostratified ciliated columnar epithelium containing goblet cells. The Rings of cartilage support the structure of the bronchi and prevent their collapse. These bronchi then divide into multiple smaller branches and ultimately become microscopic that collectively called bronchial tree (respiratory tree) [34]. The bronchioles exhibit mucosal folds and are lined by a columnar ciliated epithelium that lacks goblet cells. The final bronchioles eventually lead to the respiratory bronchioles, which is a transition area between the conducting portions and respiratory portions of the respiratory system [35]. Cilia may be present in the epithelium of the proximal portion of the respiratory bronchiole but disappear in the distal portion. A thin layer of lamina propria, smooth muscle and an adventitia surround the terminal bronchioles [34, 35].

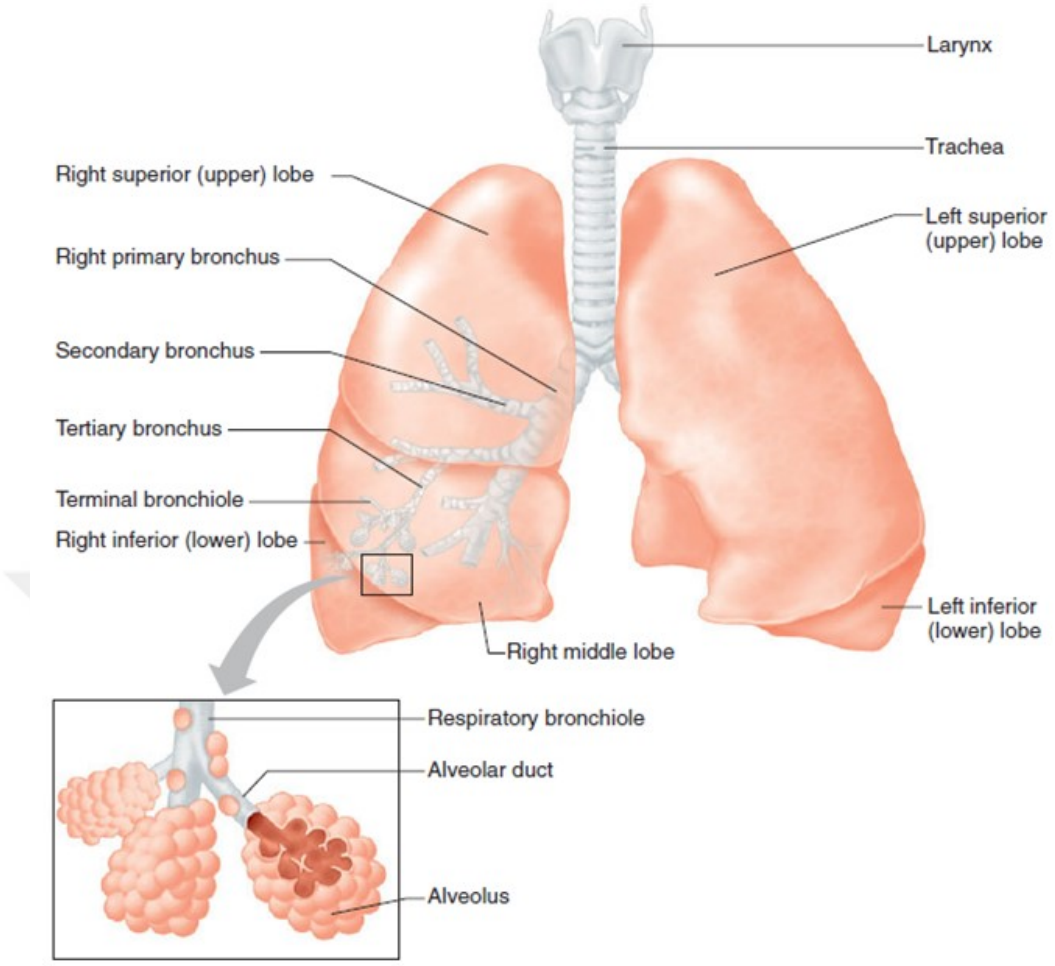

Figure 1.1. Anatomy of the lungs: bronchi, bronchioles and alveoli [34]

Each respiratory bronchiole gives rise to a tube called alveolar duct that eventually end up in clusters of microscopic air sacs termed alveoli, wherein carbon dioxide (CO2) and oxygen (O2) exchange occurs [35, 36]. The inner surface of the lungs are lined by two types of alveolar cells, alveolar type I cells (or type I pneumocytes) and alveolar type II cells (or type II pneumocytes) Figure ***1.2***.. Type I pneumocytes are simple squamous (flattened) in shape and extremely thin (~ 0.15µm). The next neighboring alveoli share a common alveolar wall and are the main sites for exchanging gases between the alveoli and blood capillaries. The cuboidal type II pneumocytes also found in the lung with fewer numbers that secret a mixture of unique and complex multifunctional substance called surfactant. The surfactant proteins cover microenvironments of the alveoli and small airways, prevent alveolar collapse at end of expiration and has an important role in innate immunity. Beside immunomodulation activity, surfactant also shows antimicrobial activity against wide-range of bacteria, fungi and viruses [36-38].

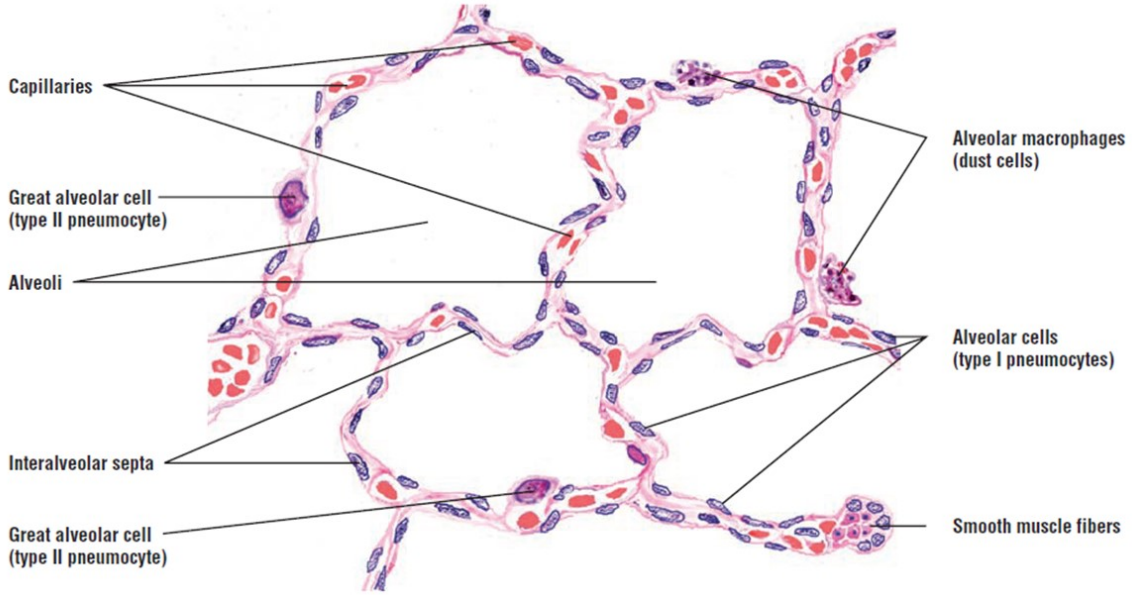

Figure 1.2. Alveolar walls and alveolar cells. Stain: hematoxylin and eosin. High magnification [39].

The lungs which extend from a portions of the bronchial tree leading to the alveoli, each of which is surrounded by extensive blood capillaries that the pulmonary artery and arteriole carry Oxygen-poor blood and the pulmonary vein and venule carry oxygen-rich blood [35]. The function of the lung, then, is to take the necessary amounts of O_2 from the atmosphere, exchanging it for CO_2 and other gaseous wastes within the alveoli and capillary bed.

The lungs, which have large surface area, about 100 m^2, inhaling nearly 20,000 Liter of air per day, continuously exposed to endogenous and exogenous oxidants (oxygen, air pollutants and cigarette, pathogens, radiation). The lungs, thus, is relatively hyperoxic state compared with cells of other organs. As such, the lung is an important target of oxidative stress from inhaled xenobiotics and toxic processes mediated by reactive oxygen species [4, 8, 9].

1.2. Biological Properties and Functionality of Ellagic acid

Ellagic acid ($C_{14}H_6O_8$) is an important natural polyphenolic component present largely as Ellagitannins (ETs) in the structure of the plant the cell membrane and cell wall and are partially responsible for their useful health impacts in several oxidation-related health conditions. Different concentrations of Ellagic acid occurs in fruits (e.g., pomegranates, persimmon and strawberries), nuts and vegetables [22, 33, 40, 41]. The total amount of ellagic acid in various fruits and plants are provided in Table **1.1**.

Table 1.1. Concentration of Ellagic acid in different plants [41].

Fruits and plants	Total EA
Bananas	2*
pear	4*
Tangerine	4*
Pineapples	6*
Plum	7*
Strawberry	31-78*
Pecan nut	33*
Walnut	59*
Raspberry	>150*
Cloudberry	>160*
Arctic bramble	>160*
Strawberry cultivar	6-34.1**
Pongamia pinnata	1.5* (bark)
	0.1* (leaves)
	0.4 (seeds)
Geraniaceae	397*
Muscadine grape cultivars	587-1900* (skin)

*,mg/100 g dry weight; **,mg/100 g frozen weight.

The chemical name of Ellagic acid is 2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]-chromene-5,10-dione. The Chemical structure of EA consists of four hydroxyl groups, the main reason for the strong antioxidant potential of EA and two lactone functional groups (Figure **1.3**.) [40, 42].

Study of bioavailability of EA from a human food source revealed that free ellagic acid is absorbed in the gut, but its bioavailability is low. ETs are solubilized to EA under physiological environment of the body, then converted to different types of urolithins by intestinal microorganism [44, 45]. These microbial metabolites are more bioavailable than ellagic acid and detected in peripheral plasma and urine [44]. Study on the bioavailability of EA in mice showed that EA is limitedly bioavailable [42]. In another study, Smart et al. [46] reported on both oral and intravenous administration of ellagic acid in mice. The authors indicated that there is no ellagic acid detected in the blood and lung, or liver tissue after oral ingestion, whereas, after intravenous administration, about 70% of the administered ellagic acid is excreted as free form and its conjugates. Various studies were reported on the bioavailability of ellagic acid and the urolithins in human after oral ingestion of pomegranate juice and ETs in healthy human volunteers. [42, 44, 47-50]. The authors showed that ellagic acid from the extracts is detected in plasma samples between 0.5 and 6 hand reach maximum concentration (31.9 ng/ml) 1 h after consumption and the EA-derived metabolites detected in human plasma two hour after pomegranate product ingestion, reaching maximum concentrations at between 24 h and 48 h. In addition, the urolithins are present in plasma and urine for up to 72 h after pomegranate juice ingestion, in free and conjugated forms

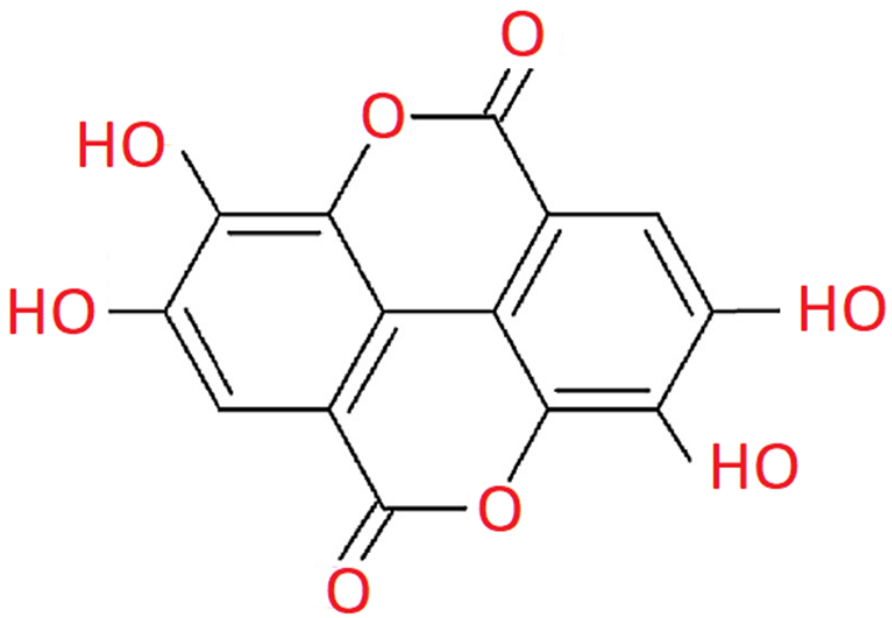

Figure 1.3. Chemical structure of ellagic acid ($C_{14}H_6O_8$) [43].

in some, but not all, volunteers. Ellagic acid, therefore, has been suggested to be absorbed into the circulation in the upper digestive system as ellagitannins hydrolyzed into free ellagic acid and may also undergo sustainable absorption in the colon as its metabolized by microorganisms into urolithins and it is rapidly cleared in the bloodstream [44, 51]. The limited bioavailability of free EA in plasma had been attributed to various factors such as type of ellagic acid precursor, its ionization at physiological PH, low solubility of free ellagic acid in gastric environments and might also be due to its excessive metabolic conversion and degradation before absorption. It has also been noted that ellagic acid is irreversibly bound to cellular DNA and proteins, which may also account for its limited transcellular uptake. Moreover, in vivo–in vitro study found that there is a large interindividual variability (genetic polymorphisms) in the absorbability of EA in human [52].

It has been indicated that EA has potential to suppress oxidative stress in biological systems. These effects are due to its antimutagenic, antioxidant and anti-inflammatory activity in experimental animals and microorganisms. It's also proposed that EA combats oxidative stress by enhancing the activity of specific biological antioxidants, antioxidant enzymes and downregulating specific genes accountable for alteration in normal biological systems [22, 40]. EA has been known for its potential anti-cancer effect via modulation of metabolism of environmental toxins; It is also reported that ellagic acid have antimutagenic activity by inhibiting the direct binding of environmental carcinogens to the DNA [22] Ellagic acid attenuated pulmonary toxicity as a result of bleomycin and cyclophosphamide administration in Wistar rats, thereby prevented lungs from harmful effect pulmonary toxicity of anticancer drugs [28]. Being a powerful antioxidant, EA mitigated the harmful effect of Carbon tetrachloride, hydrogen peroxide, D-galactosamine, scavenges hydroxyl radicals (•OH) and superoxide anion (O_2•-) [18, 42, 53]. EA decreased lung tissue damage in rats by reducing oxidative stress in experimental obstructive jaundice [20]. Furthermore, EA has been shown to provide better protection than vitamin E succinate against oxidative damage in embryonic and placental tissue in C57BL/6 J mice [54].

The existing experimental studies suggests that EA is worth testing as a potential chemotherapeutic compound against human diseases. Its protective effect on oxidative stress seems to be promising in the prevention/treatment of oxidative linked diseases. No negative reactions have been noted with the oral consumption of EA [42, 43].

1.3. Carbon Tetrachloride

Carbon tetrachloride is a hepatotoxic compound used experimentally to induce oxidative stress and to cause tissue damage by generating free radicals to mimic pathophysiological conditions in various organs including lungs [12-14]. The administration of CCl_4 induce transient

tissue disorders and possibly due to formation of the trichloromethyl radical (CCl_3 •). CCl_4 is metabolized in the endoplasmic reticulum by enzymatic reduction of cytochrome P-450 to trichloromethyl radical (CCl_3•) and if oxygen available, rapidly form a highly reactive trichloromethyl peroxyl radical (CCl_3OO•). These reactive free radicals formed by CCl_4 can induces an adverse reaction and causes damage to biological molecules such as lipids, proteins and nucleic acids [15, 16].

1.4. Apoptosis

Apoptosis is the normal physiological process that eliminates unwanted or useless individual cells without damaging neighboring cells or triggering inflammatory response. The cell death proceeds by apoptosis quickly completed within a few hours and plays a vital role during development, maintain tissue homeostasis during repair and other normal biological processes. Apoptosis occurs through distinct biological hallmarks including cell shrinkage but do not lyse, nuclear DNA fragmentation and membrane blebbing that lose their asymmetry and ability to attach to neighboring cells in a tissue. Apoptotic cells are safely sealed within dying cells until swiftly engulfed by phagocytic cells (Figure 1.4.). This process prevents the release of inflammatory factors and reducing the risk of inflammation from the cell death. otherwise, cells infected by viruses or bacteria undergo cell autonomous necrosis, which often the necrotic cells increase in volume, lyse (burst) and cellular components are released and inducing a potentially damaging inflammatory response. The necrotic process is completed within several days [55, 56].

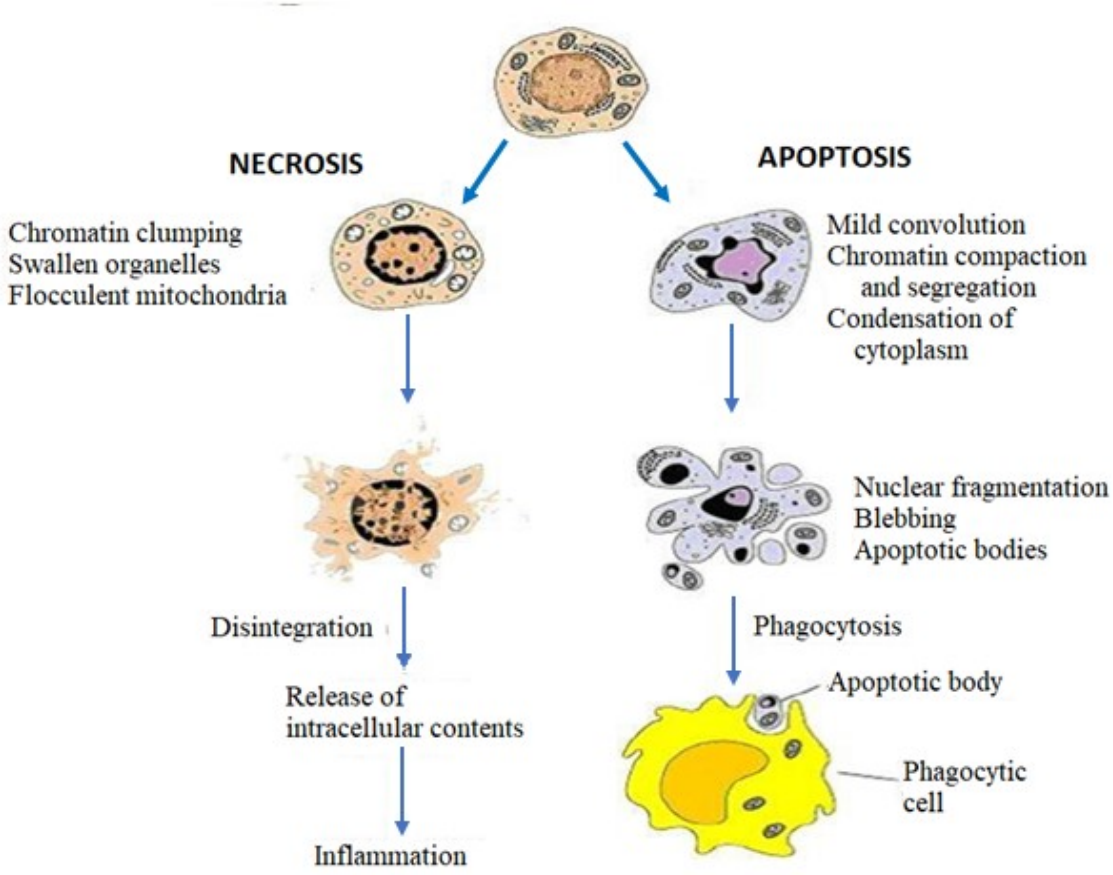

Figure 1.4. Characteristics of the apoptotic and necrotic cell death [57].

There are two main pathways mediate apoptosis, intrinsic pathway and extrinsic pathway as illustrated in Figure 1.5. The intrinsic pathway also called mitochondrial pathway triggered by internal apoptotic signal such as cytotoxic drug, oxidant and growth factor deprivation. The extrinsic pathway is activated by binding specific ligand to a death receptor. The best characterized ligand and their corresponding receptors include FAS/FASL and TNF- α /TNF receptor 1(TNFR1). The two pathways of apoptosis is mediated by specific sets of caspases that act in cascades, at the end of which caspase 3 or 7 is responsible for killing the cells [58, 59]. Apoptosis is as vital for tissue repair as cell division and differentiation. Disturbance in pathways that regulate apoptosis may leads cancers, autoimmune diseases and neurodegenerative disorders [56]. The increased apoptotic cells than “normal” tissue documented in various lung diseases including COPD, ALI, emphysema, lung cancer, fibrotic lung processes and pulmonary hypertension [58, 60].

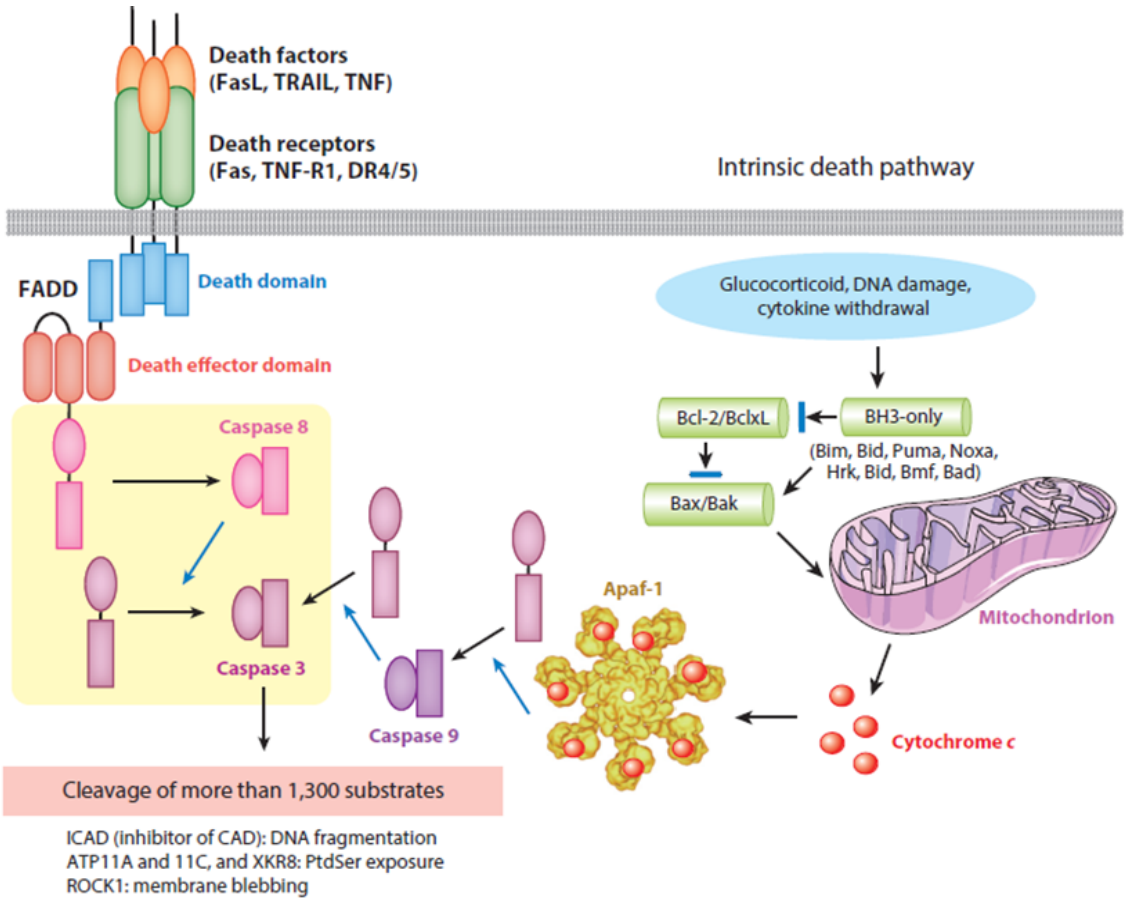

Figure 1.5. Two distinct apoptotic signaling pathways converge [55].

1.4.1 The Role of Caspase-3 in Apoptotic Cell Death

Caspase-3 is a member of the cysteine proteases (caspase) family that promote cell death. It is initially produced as zymogen and is activated by proteolytic cleavage of caspase -8 and caspase-9 in the extrinsic and intrinsic pathways of apoptosis, respectively. Thus, it serves as a point of convergence for extrinsic and intrinsic signaling pathways (Figure 1.5.). Enzymatic activation of caspase-3 leads to cleavage more than 1,300 cellular substrates to execute the apoptosis. Therefore, detection of active caspase-3 has been studied as a significant biomarker of the cell's entry point into the execution stage of apoptosis [25, 55, 61-63].

1.4.2 The Role of Bcl-2 as Pro-Survival Protein in Apoptosis

Bcl-2 is a protein member of the B-cell lymphoma-2 family proteins that regulate apoptotic cell death. this family protein regulates apoptotic cell death through either pro- or anti-apoptotic activities. Bcl-2 is the pro-survival member of the family reside in the outer mitochondrial

membrane and inhibit BAX and BAK, thereby inhibiting the release of cytochrome c to the cytoplasm, the key step in mitochondrial apoptotic pathway. Therefore, Bcl-2 have been studied as an important biomarker of apoptosis [64-66].

1.5. NF-kB

NF-kB is an oxidative stress-sensitive transcription factor, regulates more than hundred genes that control wide variety of biological effects such as inflammation and apoptosis [2, 67-69]. The nuclear factor kappa-B is composed of a heterodimer with one 50 kDa (p50) and one 65 kDa (p65) polypeptide [3].The activity of NF-kB is strictly controlled by interaction with inhibitory IkB proteins. Thus, in most cells, NF-kB is present in the cytoplasm as an inactive, IkB-bound complex. There are canonical, non- canonical and possibly atypical, pathways for the activation of NF-kB as illustrated in Figure 1.6.

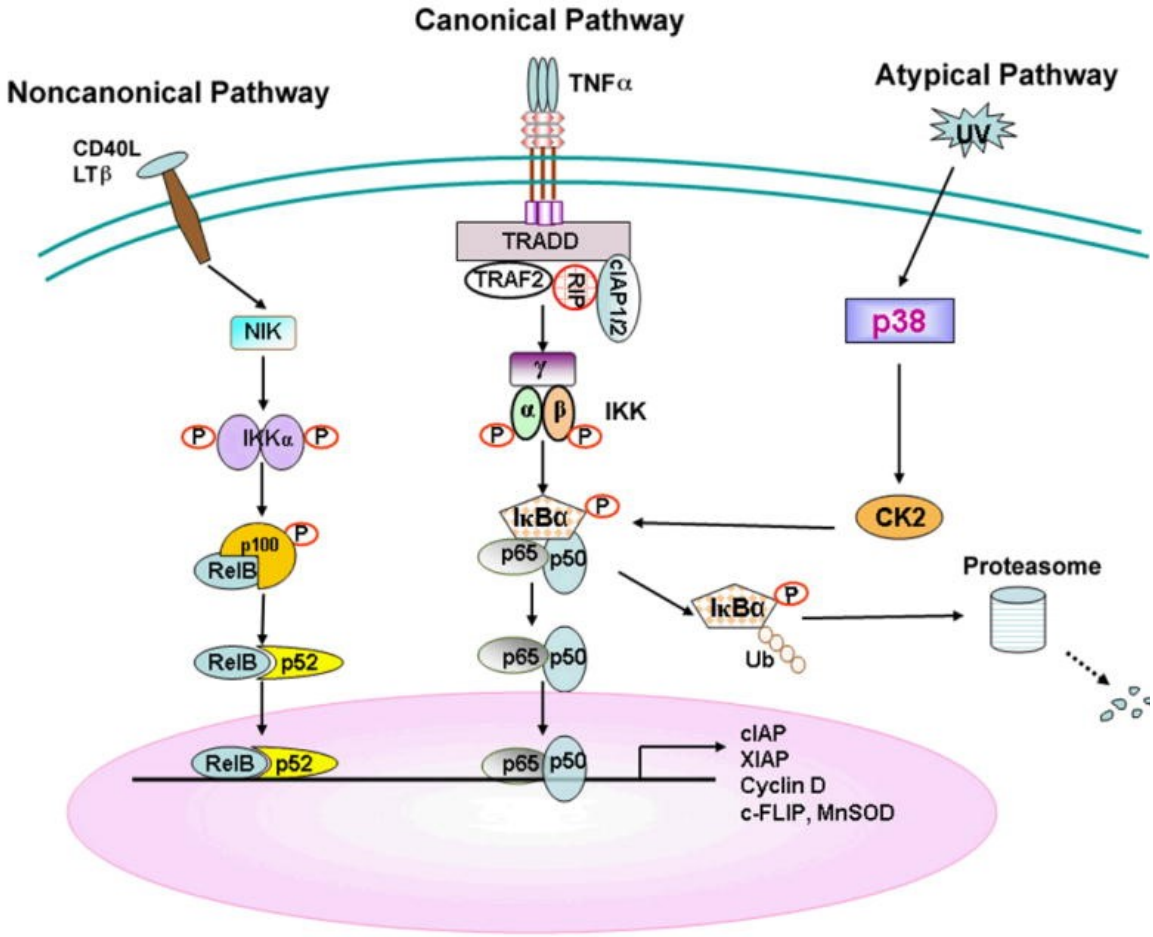

Figure 1.6. NF-kB signal transduction pathways [70].

The common upstream regulatory step in the first two best-known pathways is activation of an IkB kinase (IKK) complex, which consists of catalytic kinase subunits (IKKa and/or IKKb) and a scaffold, sensing protein called NF-kB essential modulator (NEMO). In response to different types of stimuli, IKB kinase becomes active and induce the phosphorylation and degradation of the cytoplasmic inhibitor, IkB inhibitor. Subsequently, this portion is separated from the newly activated NF-kB. Then, the activated NF-kB translocates into the nucleus, binds to specific DNA sites and up-regulate the transcription of specific target gene [67, 71, 72]. It is well known that highly conserved transcription factors NF-kB regulates the expression of numerous genes responsible for inflammation, apoptosis and proliferation. Several evidence established that NF-kB expression has been associated with the pathogenesis of ALI and it up-regulates the transcriptions of TNF-α and COX-2 [73, 74].

1.6. TNF-α

TNF-α is the most commonly studied pro-inflammatory cytokine of the TNF superfamily. Overproduction of TNF-α associated with the pathogenesis of numerous inflammatory diseases of the lung. In addition to its fundamental role in the inflammation, there is a growing evidence implicating TNF- α in the cellular toxicity [75]. It has been reported that releasing TNF-α by activated macrophage caused necrosis of tumors in vivo and cytolysis or cytostasis of certain transformed cells. Therefore, pharmacological drugs that can either reduce or block TNF production may have beneficial effects in a wide range of diseases [76].

The TNF-α signaling through TNF receptors (TNFR1 and TNFR2) can activate different pathways to induce apoptosis, cell survival or inflammation, as illustrated in Figure 1.7 [77]. Over-expression of TNF-α results in the development of inflammatory reactions which are characteristic phenomenon of many pulmonary diseases including, asthma, chronic bronchitis, COPD, ALI and acute respiratory distress syndrome (ARDS) [76, 78].

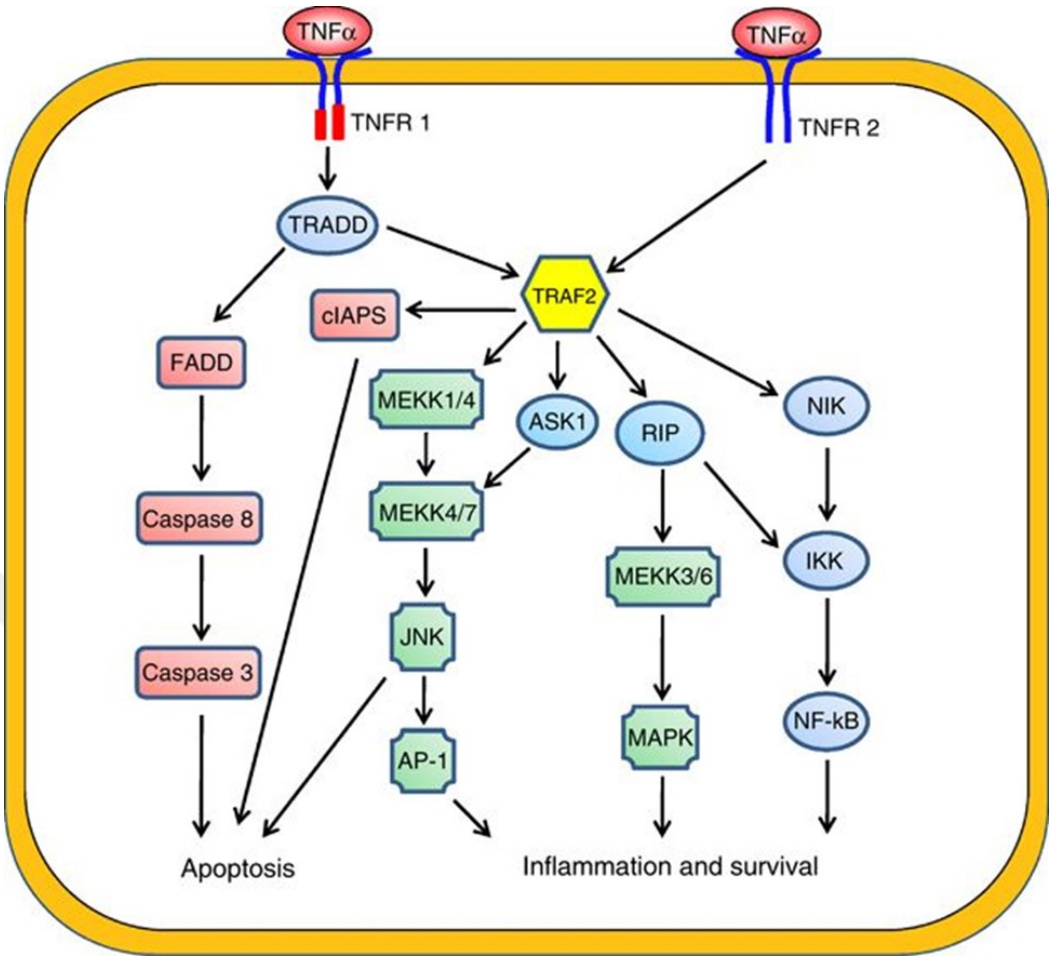

Figure 1.7. The downstream signaling pathways of TNF-α [77].

1.7. COX-2

COX-2 is the inducible isoform of cyclooxygenases, which is regulated by the transcriptional factor NF-kB, growth factors and different proinflammatory cytokines such as IL1β, IL6 or TNF-α. Therefore, overexpressed during inflammation. The induction of COX-2 is involved with an increased biosynthesis of prostaglandin, which Modulates cell proliferation, cell death and invasion of tumors in many kinds of cancer, including lung cancer [79-82]. Therefore, COX-2 selective inhibitors is a potential pharmacological target for cancer prevention and treatment (Figure 1.8.) [83].

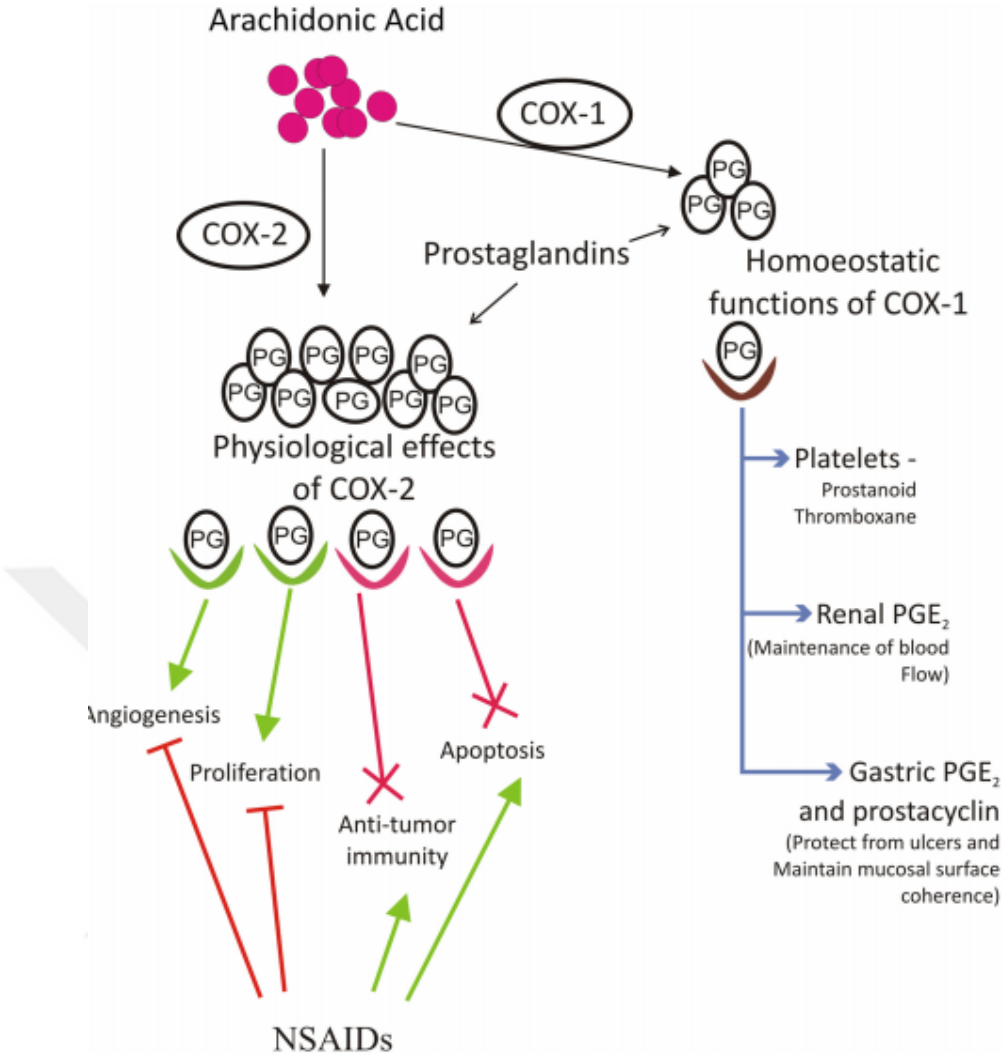

Figure 1.8. Signaling pathways mediate conversion of Arachidonic acid to prostaglandins by action of COX-1 and COX-2 enzymes. An increased level of prostaglandin associated with various biological responses [84].

1.8. Nrf-2

Nrf-2 is a transcription factor that acts as the main regulator of endogenous inducible defense systems in the body against oxidative stress [85, 86]. The best-characterized mechanism of Nrf-2 regulation is mediated by interaction with its cytoplasmic repressor, Kelch-like ECH-associated protein 1 (Keap1). Under the normal physiological conditions, Nrf-2 is primarily found in the cytoplasm bound to Keap1, Keap1 holds Nrf-2 in the cytoplasm and mediates Nrf-2 ubiquitination to facilitate its degradation by the 26S proteasome, thereby inhibits the downstream transcriptional activity of Nrf-2 under normal conditions [87]. Nevertheless, in response to oxidative stress or upon exposure to Nrf-2 activators such as free ROS and phenolic phytochemicals, the interaction between Nrf-2 and keap1 impaired, which ultimately prevents Nrf-2 ubiquitination and

degradation. The dissociated Nrf-2 translocate to the nucleus and activate cellular rescue pathways through up-regulation of antioxidant response element-bearing genes (ARE), which is encoding antioxidant defense system and cytoprotective proteins (Figure 1.9.) [85, 86]. Pharmacological and genetic studies assumed that there is interaction between Nrf-2 and NF-κB signaling pathways to regulate the transcription or function of downstream target proteins having both positive and negative effects on the target gene expression. NF-kB activate proinflammatory cytokine, COX-2. 15d-PGJ2, as a final product of COX-2 activities, induce the Nrf-2 that eventually alleviate the consequences of oxidative stress [87, 88].

The Keap1/Nrf-2/ARE signaling pathway principally regulates cellular rescue pathways in several lung diseases that involve oxidative stress, inflammation and cell fate decision [87, 89].

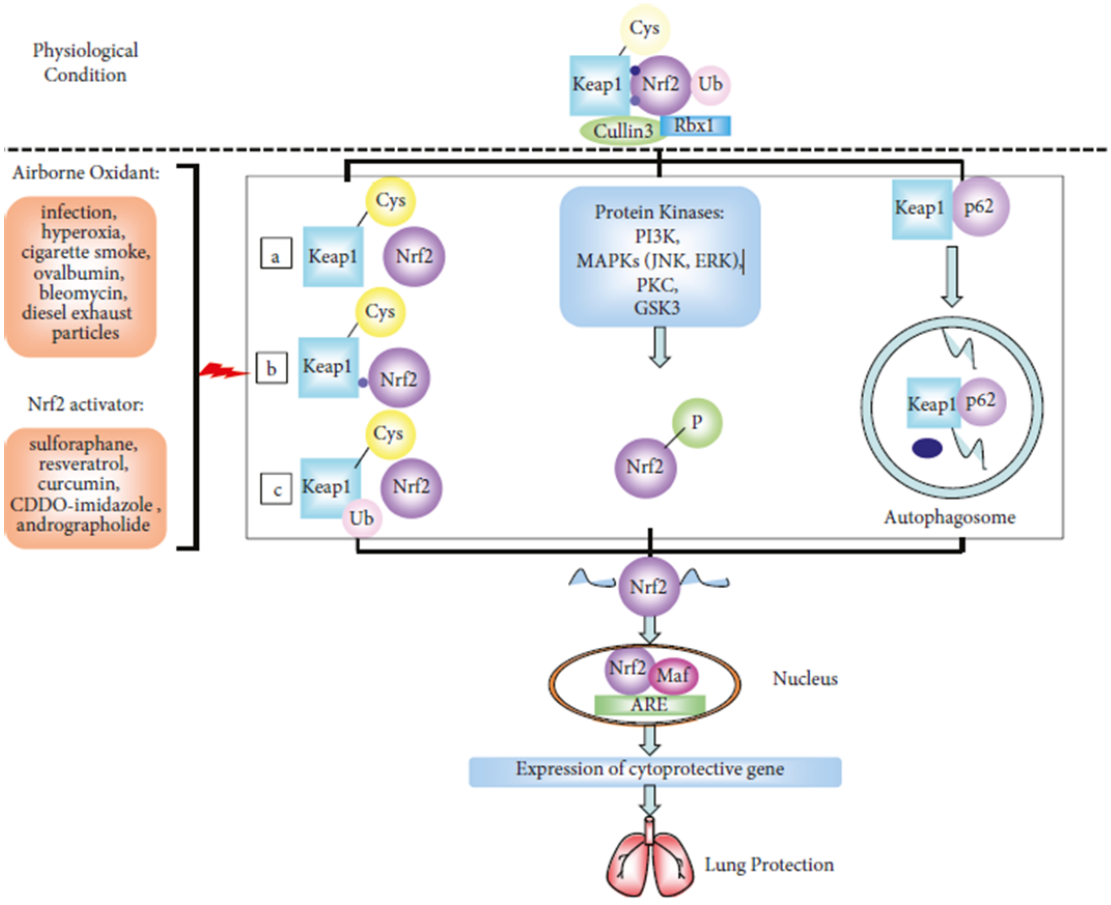

Figure 1.9. The Nrf-2 signaling pathway and Nrf-2 mediated antioxidant responses in the lungs [86].

1.9. Catalase Enzyme

Catalase enzyme, is a tetrameric heme protein and one of the major antioxidant enzymes in the lung, detoxifies hydrogen peroxide (H_2O_2) into oxygen and water. Catalase is highly efficient; it can breaks down millions of H_2O_2 molecules in one second [90]. Excessive H_2O_2 is potentially hazardous for almost all cell components, when ferrous iron (Fe_{2+}) present in the cell, can react with hydrogen peroxide to form more toxic hydroxyl radicals (•OH). The hydroxyl radical oxidize lipids and starting a free radical chain reaction that will damage the membrane. Therefore, rapid and efficient removal is vitally essential for aerobically living systems. On the other hand, H_2O_2 acts as a second messenger in signal-transduction pathways [91-93]. In the lugs, catalase activity was found to be reduced in several lung diseases such as lung cancer [94, 95] and asthma [96].

1.10. Glutathione

Glutathione is the most important tripeptide (cysteine, glycine and glutamic acid) intracellular antioxidant synthesized in most cells, the higher concentration exists in hepatocytes where scavenging free radical attack. There is a high concentration of GSH in some extracellular environments such as the epithelial lining fluid and tissue of the lung [93]. There are two types of glutathione in the cells: reduced glutathione (GSH) and oxidized form (GSSG), where two GSH moieties combine by sulfhydryl bonds. The GSH and associated enzymes are controlled by a transcription factor Nrf-2. Glutathione peroxidase (GP_x) and glutathione-s-transferase (GST) maintain a regular redox balance, basically through detoxification reactions using GSH, converting it to GSSG. Glutathione reductase (GR) drives the regain pathway by converting GSSG to GSH using NADPH and restores the cellular GSH pool [97]. Therefore, GSH and GSH-dependent enzymes are needed for maintaining the regular redox balance in the body and support in cell survival under stress conditions. These enzymes catalyze the reduction of hydrogen peroxide by reduced glutathione into water and GSSG. In the cellular environment, GSH acts as an antioxidant and protects cells against a wide range of free radicals, including ROS, lipid hydroperoxides, xenobiotic toxicants and heavy metals [97].

In the lung, decreasing GSH implicated in several pulmonary diseases and make them more susceptible to damaging effects of subsequent exposure to inhaled oxidants. In contrast, elevating intracellular glutathione levels can reduce inflammatory reaction in the lung cells by reducing NF-kB activation (Figure 1.10). Thus, the reduced glutathione is vital protective antioxidant in the lung [98].

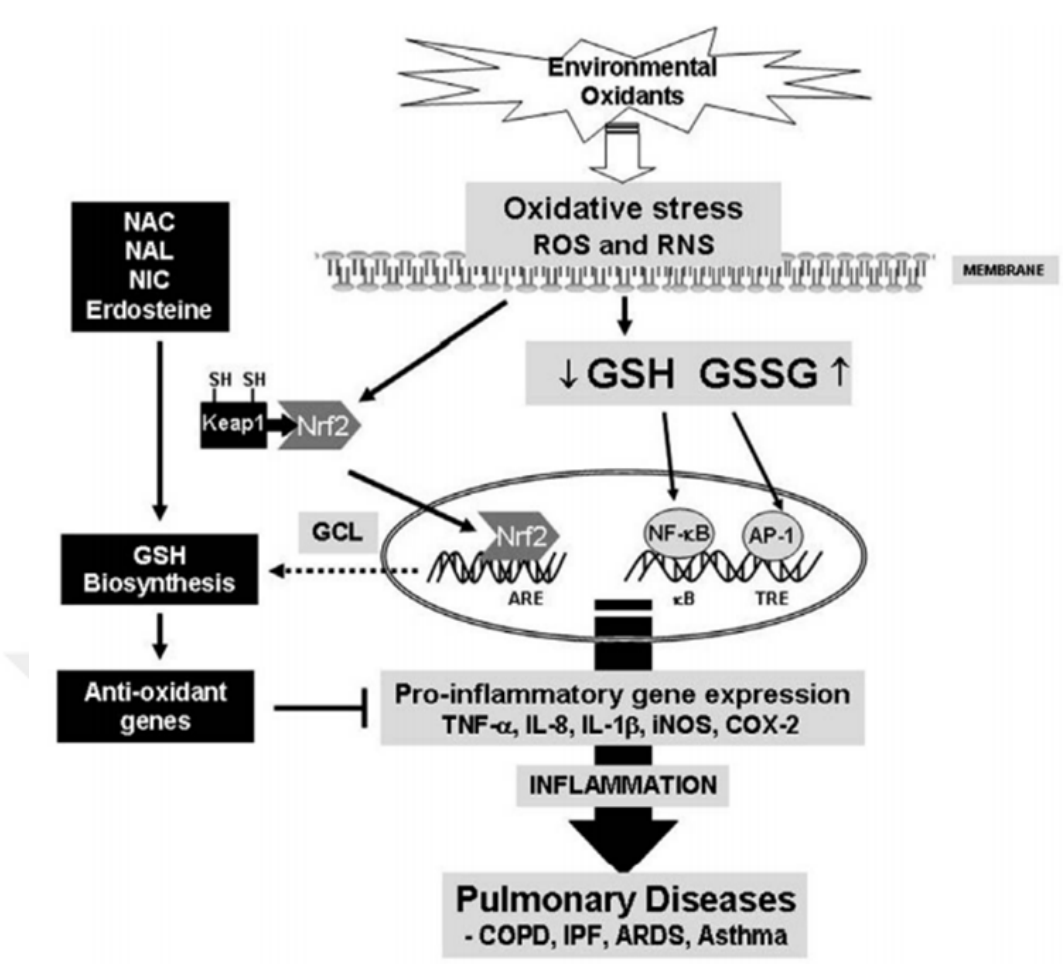

Figure 1.10. Oxidative stress induced-change in the intracellular redox ratio of GSH/ GSSG levels and redox regulation of inflammatory mediators [99].

1.11. Malondialdehyde

The main mechanism of tissue damage caused by reactive oxygen species is the peroxidation of lipids in cell membranes. The breakdown of lipid hydroperoxides results in a wide range of byproducts, one of which is MDA that may present in a free form or as a complex with different tissue constituents. MDA which is formed as a result of the oxidative degradation of polyunsaturated fatty acids, now accepted as a dependable biomarker of lipid peroxidation [100, 101]. The resulting MDA results in the cross-linking of the components of the membrane by influencing the ion exchange from the cell membranes and causes deleterious consequences such as ion permeability and the change of enzyme activity. Because of this property, malondialdehyde can react with the DNA and RNA. Therefore, MDA has mutagenic, genotoxic and carcinogenic properties [100, 102, 103].

2. MATERIALS AND METHODS

2.1. Chemical Matters and Ellagic Acid

All primary antibodies NF-kB sc-8008, caspase-3 sc-70497, beta aktin sc-47778, Nrf-2 ab137550 and Bcl-2 sc-509 and the secondary antibodies ab97023 and sc-516102 were purchased from Santa Cruz Biotechnology (Germany) and Abcam (UK). EA (A15722) were purchased from Alfa Aesar (Germany). All other chemicals and reagents were purchased from Sigma-Aldrich (Germany), Merck (USA), Bio-Rad (USA) BioShop (Canada).

2.2. Animal Materials and Research Groups

In the present study, lung tissue taken from a previous study were used with the permission of University of Firat, Animal Experiments Ethics, Committee 13.02.2019, protocol number 2019-22, meeting number 2019-03 and decision number 36. In our previous study, 36 Wistar albino rats (n = 36, 8 weeks old) were obtained. The rats were maintained at an ambient temperature of (22 ± 2 ° C) with a 12-hr light/dark cycle. The Wistar albino rats were divided into four groups, the treatment groups were as follows:

Control group: Rats were administered only standard diet.

Ellagic Acid (EA) group: Rats were administered only EA (10 mg/kg b.w., ip).

Carbon tetrachloride (CCl_4) group: Rats were administered only CCl_4 (1.5 ml/kg b.w., ip).

CCl_4 + EA group: Rats were received both CCl_4 (1.5 ml/kg b.w., ip) and EA (10 mg/kg b.w., ip).

The study continued for 8 weeks. After this time, the lung tissue was taken and used in the present study.

2.3. Carbon Tetrachloride Preparation and Applications

CCl_4 application was administered intraperitoneally (i.p.) with olive oil at a ratio of (1:3) at the dose of (1.5 ml/kg b.w.) two times a week for four weeks started from the first day when the animals were divided into 4 groups [104, 105].

2.4. Ellagic Acid Preparation and Applications

100 mg of ellagic acid were dissolved in 10 ml of dimethyl sulfoxide. Ellagic acid taken from the stock solution were administered to the rats intraperitoneally at the dose of (10 mg/kg b.w.) five times a week started after first week and continued for the end of experimental period of 8 weeks [18, 106].

2.5. Tissue Homogenization

The Lung tissues were fragmented into small pieces and disintegrated in the lysis buffer (0,5M Tris; pH: 8, EDTA, PMSF). Homogenates were prepared using mechanical homogenizer. The samples then centrifuged at 15,000 rpm for 45 min. The supernatant was stored at −80 °C for further use [18, 107].

2.6. Analysis of Proteins by SDS-PAGE and Western Blotting Technique

The primary antibodies were diluted at a ratio (1:500 dilutions of each), while the secondary antibodies diluted at a ratio (1: 1,000 dilutions of each). The SDS-PAGE technique used to separate the protein samples (TNF- α, caspase-3, Bcl-2, Nrf-2, NF-kB and COX-2) on 12% gel. Then, these proteins were blotted on nitrocellulose membrane by western blotting technique and their synthesis rates were examined [108]. The protein antibody complexes levels were detected by the density measurement analysis system (Image J; National Institute of Health, Bethesda, USA).

2.7. Determination of Catalase Enzyme Activity

In order to be used in the catalase measurement, concentrated hydrogen peroxide (H_2O_2) were added to the 1/15 M Na-K-phosphate buffer (Na_2HPO_4-KH_2PO_4) pH 7 solution until the absorbance at 240 nm wavelength in the spectrophotometer become 0.7-0.9. 1,000 microliters of this mixture prepared to measure the catalase activity of the samples were put in the incubator. depending on the working range, supernatant were added starting from increasing concentrations of the 30 microliters and then the absorbance change of H_2O_2 was recorded on the spectrophotometer for 30 sec. The number of enzyme units in ml were calculated from the difference in optical density read [18, 109].

2.8. Determination of GSH Level

The GSH content from lung homogenate were measured spectrophotometrically based on the reaction of GSH with DTNB (also known as Ellman's reagent) [110]. Briefly, 0.1 ml cell homogenate were added to 0.4 ml of TCA (10% trichloroacetic acid) solution. After vortexing thoroughly and centrifugation at 3,000 rpm for five min, 0.1 ml of the supernatant were taken into a clean tube and mixed with 0.9 ml of distilled water, 2 ml of Tris buffer (0.4M pH 8.9) and 0.1 ml of DTNB solution. The resulting yellow color were read at 412 nm wavelength in spectrophotometer against distilled water [111].

2.9. Determination of Malondialdehyde level

Lung tissue specimens were divided into small pieces and disintegrated in the mechanical homogenizer in a manner of 4.5 ml of 1.15% KCl per 0.5 grams of tissue. From this prepared homogenization, MDA determination as the final product of lipid peroxidation were made according to the method described by Ohkawa et al. [112]. This method is based on the reaction of malondialdehyde from aldehyde products of lipid peroxidation and thiobarbutric acid (TBA). During the measurement process, a mixture of (0.1 ml 8.1% sodium dodecyl sulfate (SDS) + 750 µl 20% (pH 3.5) acetic acid solution) were added to 0.1 ml tissue homogenate. Then, 750 µl of 0.8% (pH 3.5) thiobarbutric acid solution added with distilled water to become final volume of mixture 4 ml. This mixture was left at 95 °C in a boiling water bath for 45 min and then cooled and vortexed following the addition of a mixture of 1 ml of distilled water and a mixture of 5 ml of 15:1 ratio of (v/v) n-butanol-pyridine. After centrifugation at 5,000 rpm for 10 min, supernatant were taken and measured spectrophotometrically at a wavelength of 532 nm. Results was recorded in nmol/g [18, 112].

2.10. Statistical Analysis

The data from individual experiments have been evaluated by using variance analysis in SPSS 21 package program. One-Way ANOVA Post Hoc test were applied to determine the differences among the groups. For the reliability of the statistics, the measurements were made at least three replications. Then the evaluation process was started.

3. RESULTS

3.1. Effects of EA on Lung Tissue Catalase Enzyme Activities

In the present findings, Catalase enzyme activities significantly decreased in CCl_4 group compared to other groups. Treatment with EA significantly ($p < 0.05$) increased catalase enzyme activity in EA and EA+CCl_4 group compared to CCl_4 group. The highest level of catalase activity noticed in EA group, the lowest catalase enzyme activity noticed in CCl_4 group (Table 3.1., Figure 3.1.).

Table 3.1. Lung tissue catalase activity results

Groups	Lung tissue catalase activity (U/mg protein)
Control	79.13 ± 0.02^{a}
EA	79.87 ± 0.03^{a}
CCl_4	45.46 ± 0.03^{c}
EA +CCl_4	58.75 ± 2.51^{b}

a-c: Differences between groups with different letters are statistically significant ($p < 0.05$). One-Way ANOVA Post Hoc Games-Howell Test.

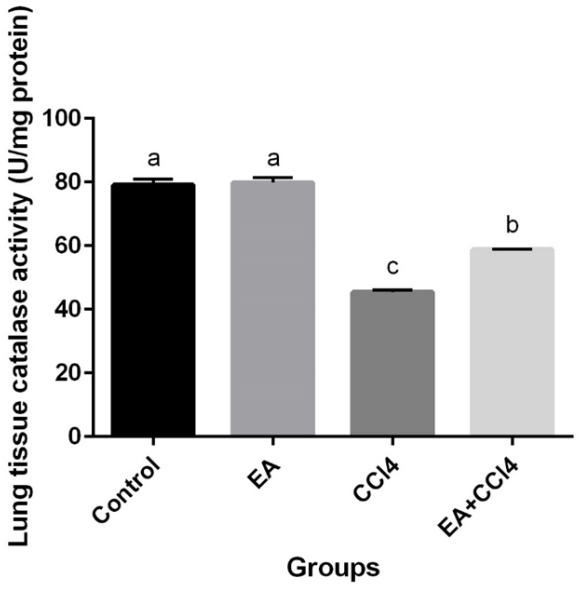

Figure 3.1. Shows the effect of EA on catalase enzyme activities in CCl_4 induced lung damage in rats. different letters indicate statistically significant difference between groups. Values are statistically significant at $p < 0.05$. One-way analysis of variance (ANOVA) Post Hoc LSD Test.

3.2. Effect of EA on Lung Tissue GSH Levels

The levels of GSH content represented in Table 3.2 and Figure 3.2. Administration of CCl4 significantly ($p < 0.05$) declined GSH level compared to control group and EA group. EA treatment significantly restored the depleted GSH level in EA and EA+CCl4 group.

Table 3.2. Lung tissue GSH results

Groups	Lung tissue GSH (µmol/mg protein)
Control	33.67 ± 0.02^{a}
EA	34.86 ± 0.02^{a}
CCl_4	19.26 ± 0.5^{c}
EA +CCl_4	27.96 ± 0.02^{b}

a-c: Differences between groups with different letters are statistically significant ($p < 0.05$). One-Way ANOVA Post Hoc Games-Howell Test.

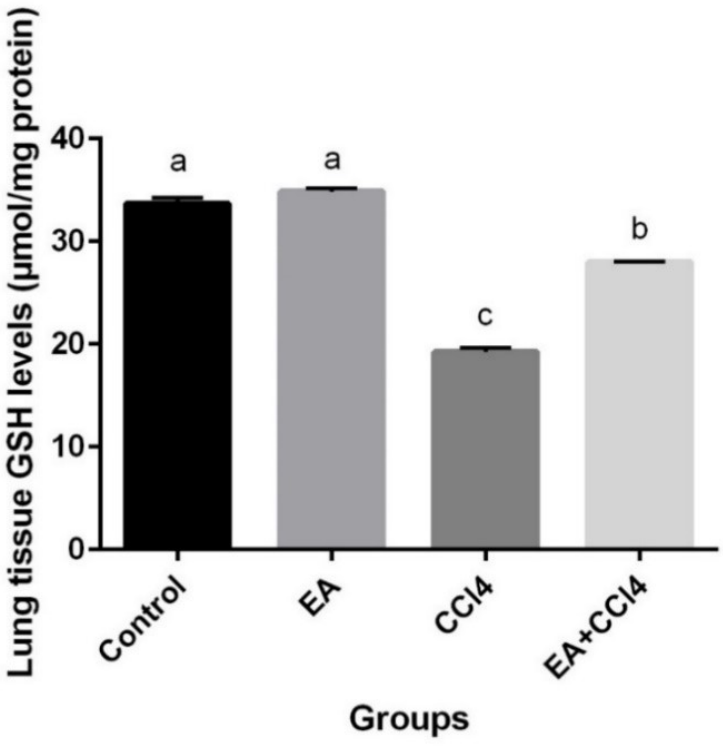

Figure 3.2. Shows the effect of ellagic acid on GSH levels in CCl_4 induced lung damage in rats. different letters indicate statistically significant difference between groups. Values are statistically significant at $p < 0.05$. One-way analysis of variance (ANOVA) Post Hoc LSD Test.

3.3. Effect of EA on Lung Tissue MDA Levels

The values in Table 3.3 and Figure 3.3 shows that the level of MDA decreased in the ellagic acid group and CCl_4 + EA group compared to the CCl_4 group. The highest levels of MDA were noticed in CCl_4 groups ($p < 0.05$).

Table 3.3. Lung tissue MDA results

Groups	Lung tissue MDA (nmol/g)
Control	3.67 ± 0.03^{c}
EA	3.75 ± 0.03^{c}
CCl_4	9.61 ± 0.02^{a}
EA +CCl_4	6.88 ± 0.25^{b}

a-c: Differences between groups with different letters are statistically significant ($p < 0.05$). One-Way ANOVA Post Hoc Games-Howell Test.

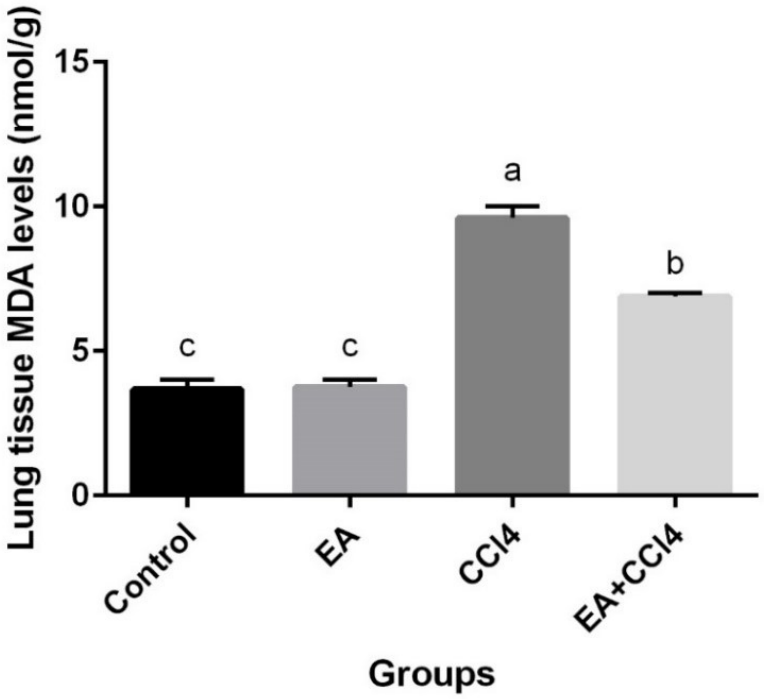

Figure 3.3. Shows the effect of ellagic acid on lung tissue MDA in CCl_4 induced lung damage in rats. different letters indicate statistically significant difference between groups. Values are statistically significant at $p < 0.05$. One-way analysis of variance (ANOVA) Post Hoc Games-Howell Test.

3.4. EA Decreased the Expression Levels of Bcl-2, NF-kB, COX-2, TNF- α and Induced Nrf-2 and Caspase-3 Proteins Expression

The level of the pro-apoptotic protein, Caspase-3 and the pro-survival protein, Bcl-2 are shown in Figure 3.4A and Figure 3.4B, respectively. Results of Caspase-3 showed a significance differences between the groups. In CCl_4 treatment rats, Caspase-3 levels significantly reduced compared to control group. Ellagic acid treatment significantly elevated the Caspase-3 levels in EA groups compared to other groups. The Bcl-2 protein levels significantly increased ($P < 0.05$) in the CCl_4 group compared to other groups, treatment with EA significantly decreased the anti-apoptotic protein Bcl-2 compared to CCl_4 group, however, there is no statistically significance difference ($p > 0.05$) between ellagic acid only group and control group. Figure 3.4C shows the effects of EA on lung tissue gene expression levels of NF-kB. In the CCl_4 group, carbon tetrachloride caused a significant increase ($p < 0.05$) in lung tissue expression levels of NF-kB compared to the control group. Administration with EA led to a significant reduction ($p < 0.05$) in lung tissue gene expression levels of NF-kB compared to CCl_4 group, whereas no statistically significance difference ($p > 0.05$) were observed between the control group and the ellagic acid only group regard to NF-kB. When Figure 3.4D examined, the COX-2 protein expression levels differed at a statistically significant ($p < 0.05$) between all groups. The highest value was detected in the CCl_4 group, while the lowest value was detected in the EA group. TNF-α protein expression from the lung tissue homogenate of all experimental groups are shown in Figure 3.4E. TNF-α protein expression levels were significantly ($p < 0.05$) increased in CCl_4-treated rats when compared with the other groups, administration of EA significantly attenuated this change, whereas no statistically significance difference ($p > 0.05$) were observed between the control group and the ellagic acid only group. Effects of EA treatments on lung tissue expression of Nrf-2 protein is presented in Figure 3.4F. The Nrf-2 protein expression significantly decreased in CCl_4 group to a lowest value compared to other groups. Administration of EA significantly increased the Nrf-2 protein expression in Ellagic acid group compared to CCl_4 group.

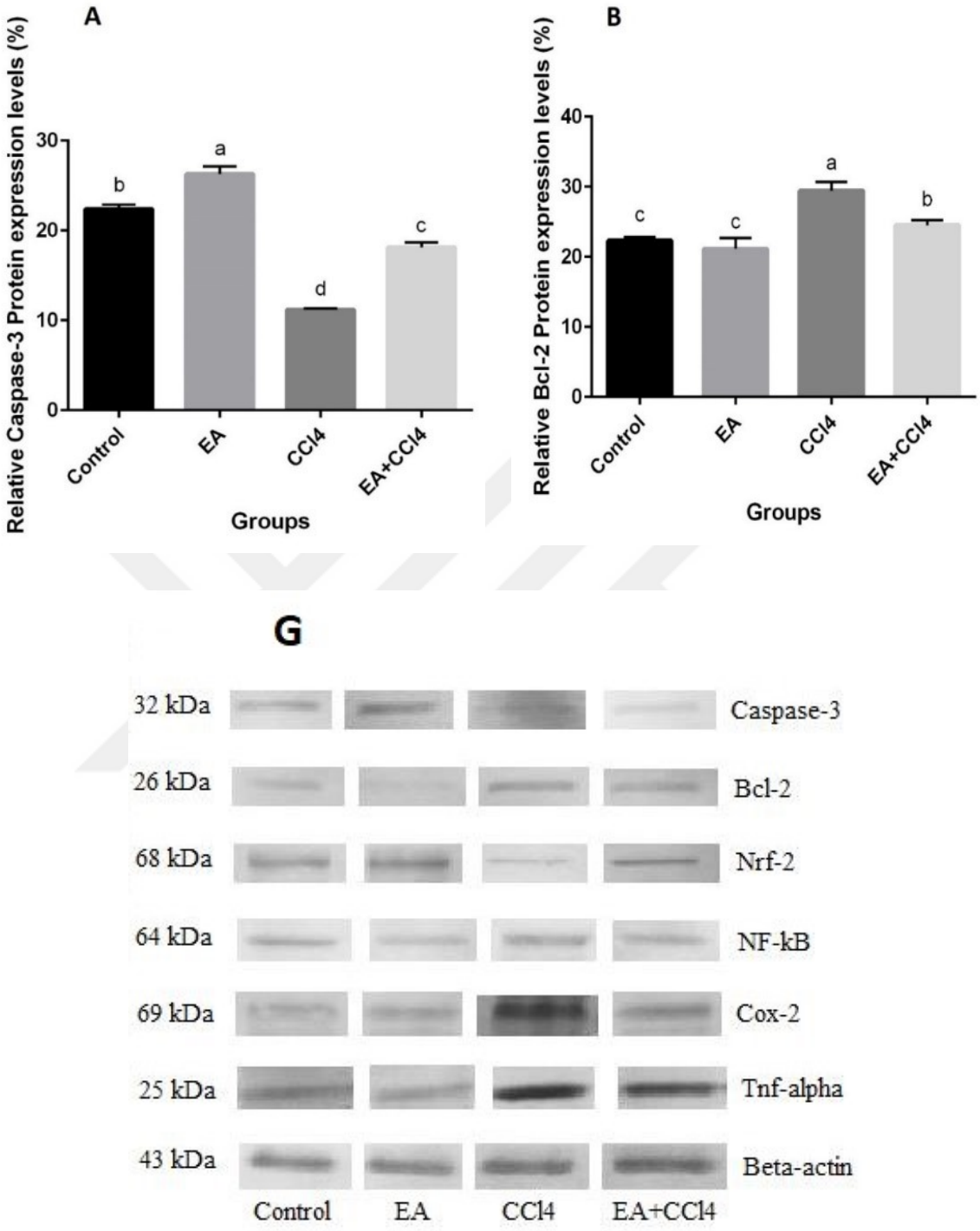

Figure 3.4. Western blotting mean protein expression results; A: caspase-3, B: Bcl-2, C: NF-kB, D: COX-2, E: TNF-α and F: Nrf-2. a-f: different letters indicate statistically significant difference between groups. Values are statistically significant at $p < 0.05$. One-way analysis of variance (ANOVA) Post Hoc Duncan Test, G: western blotting protein bands.

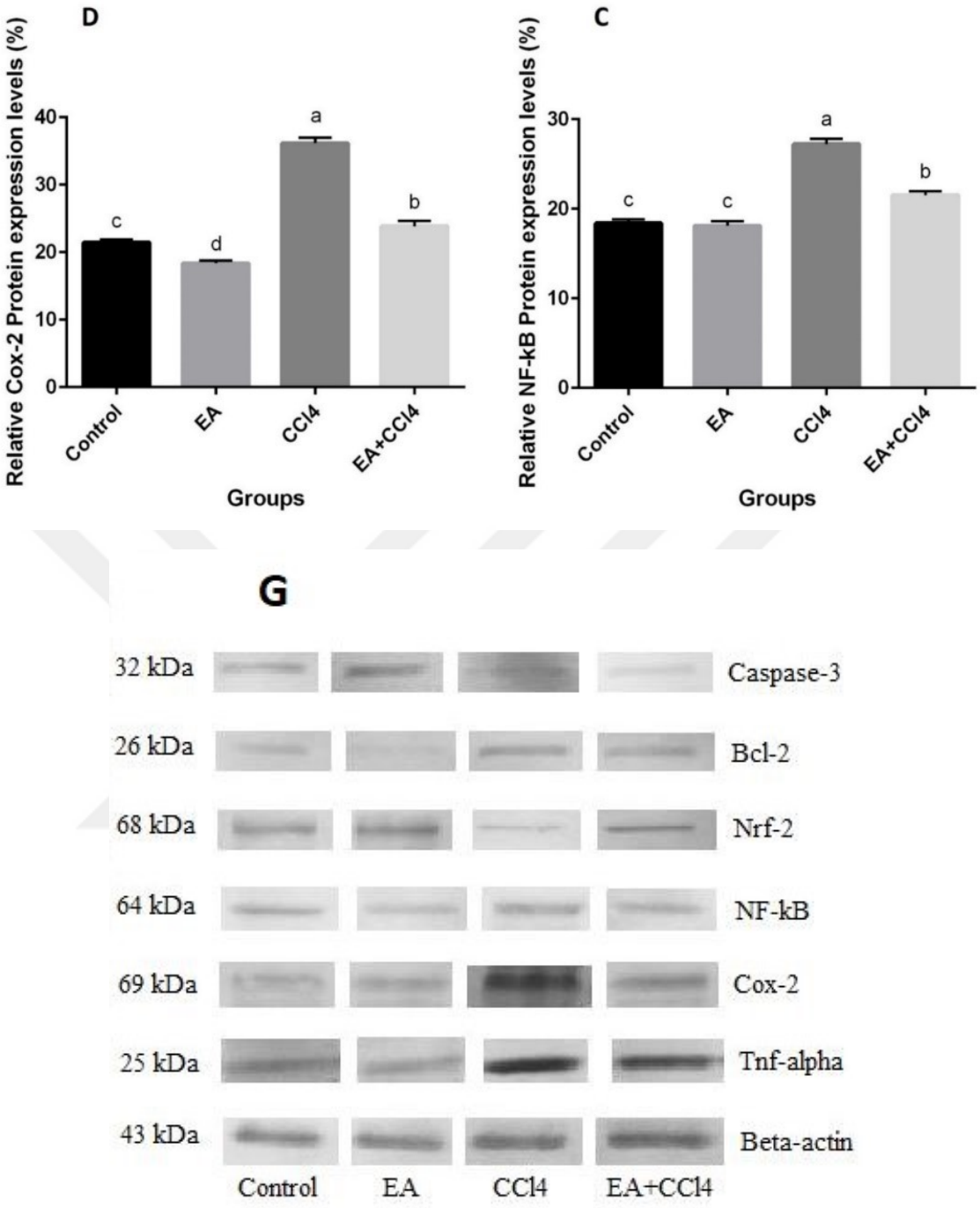

Figure 3.4. (Continued)

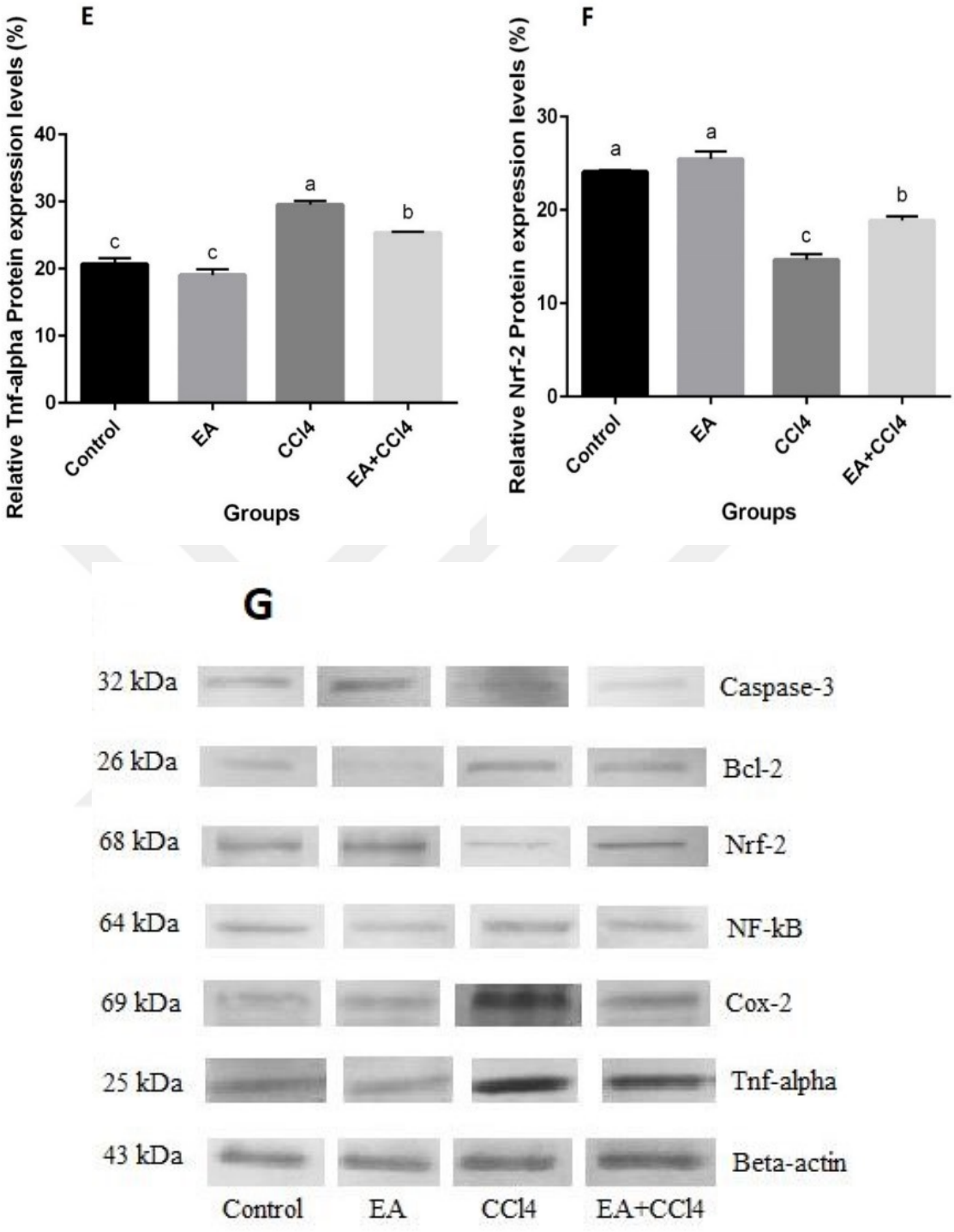

Figure 3.4. (Continued)

4. DISCUSSION

In this experimental study, several aspects related to the protective effects of ellagic acid, a polyphenolic compound, against CCl_4-induced lung damage were investigated using several proteins, cytokines and transcriptional factors. In this study, ellagic acid demonstrated therapeutic effect against lung damage through increasing antioxidant activity and/or expression, preventing lipid peroxidation, decreasing inflammatory reactions and apoptotic-inducing activities.

Inflammation is crucial for maintaining homeostasis of the tissue and protection against infections; however, uncontrolled and prolonged inflammation increases the risk of cancer and tissue damage in various organs, a characteristic feature of several inflammation-related-lung diseases [45, 113, 114]. NF-κB, a major transcription factor, serve as a pivotal mediator of inflammatory responses and regulates the expression of numerous genes responsible for apoptosis and proliferation. Several evidence established that NF-κB expression has been associated with the pathogenesis of ALI and it up-regulates the transcription of TNF-α and COX-2 [73, 74]. Ellagic acid has been found to inhibit the production of inflammatory mediators via downregulation of NF-kB in vivo and in vitro [33]. Gu et al. [115] studied the effects of EA in experimentally-induced liver injury in mice. The authors reported that EA inhibited NF-κB protein expression. Zhou et al. [116] studied the effect and mechanism of action of EA in an ovalbumin-induced asthma model in mouse. The authors indicated that EA attenuates the inflammatory reactions in ovalbumin-induced asthma in mice, likely through inhibiting NF-κB activation. In the present study, NF- κB expression significantly ($p < 0.05$) decreased in the EA treated group compared to the CCl_4 + EA and CCl_4 group. These findings suggest that production of cytokines and pro-inflammatory mediators by NF-κB response genes could be represent the degree of inflammation and lung damage in CCl_4-induced rats [74]. Thus, anti-inflammatory effects of EA could be related to the intervention of NF- κB pathways in CCl_4-induced lung damage in rats, which is one of the most important mechanisms to prevent lung damage by EA.

Overexpression of COX-2, an inflammation-associated enzyme, associated with inflammation and implicated in the pathogenesis of various malignancies including lung cancer, where it related with a wrong prognosis [74, 79, 117-119]. Major anti-carcinogenic effects of nonsteroidal anti-inflammatory drugs suppress lung tumorigenesis through both the induction of apoptosis and inhibition of COX-2 expression [120]. EA administration decreased the expression of COX-2 in experimentally-induced colon carcinogenesis [74]. El-Shitany et al. [32] studied the protective role of EA on carrageenan-induced acute inflammation. The authors reported that EA ameliorated COX-2 expression, reduced the levels of NF-κB mRNA expression and significantly

decreased paw TNF-α formation. In addition, the authors also reported that EA reduced MDA formation and restored the depleted GSH levels in the paws. In another study, Karlsson et al. [121] also noted that ellagic acid suppressed the increased protein expression of COX-2 in lipopolysaccharide-induced human monocytes. Attilio et al. [122] suggested that EA may have a nonspecific COX inhibitory effects similar to those of ketorolac in male Sprague-Dawley rats. In the present study, EA substantially ($p < 0.05$) decreased expression of COX-2 in EA group and EA + CCl_4 group compared to CCl_4 group. Thus, these results support that use of ellagic acid is beneficial in the treatment of lung injury through reduction of inflammation and cancer marker COX-2.

Induction of pro-inflammatory cytokine, TNF-α, results in the development of inflammatory reactions, which are characteristic phenomenon of many pulmonary diseases including, asthma, chronic bronchitis, COPD, ALI and ARDS [76, 78]. Zhang et al. [123] studied the possible roles of TNF-α in the pathogenesis of pulmonary fibrosis. The authors observed that treatment with Anti-TNF-α antibody caused a significant decrease in lung fibrosis through decreasing the lung eosinophils and eosinophil-derived cytokines without effect on the number of lung macrophages, suggested a possible role of TNF-α in magnifying the inflammatory responses and development to pulmonary fibrosis. In addition to its fundamental role in the inflammatory conditions, there is a growing evidence that TNF-α causes cytolysis of certain transformed cells [76, 78]. Substantial evidence has shown that TNF-α gene expression is associated with inflammatory and immune responses in a manner that is dependent on the NF-kB activation [124, 125]. Gu et al. [115] reported that lipopolysaccharide/d-galactosamine increased hepatic MDA content and serum and liver TNF-α levels. However, the authors observed that treatment with EA significantly reduced the increase of liver malondialdehyde level and significantly reduced serum and liver TNF-α levels. Thus, this cytokine, along with other proinflammatory compounds, forms a positive auto-regulatory loop that initiates, amplifies and perpetuates the inflammatory responses, a characteristics of various chronic inflammatory lung diseases. Therefore, inhibiting TNF-α release in the lungs might be an effective way for the treatment of inflammatory lung diseases [73, 126]. In this study, we found that EA efficiently ($p < 0.05$) decreased the expressions of TNF-α in CCl_4-induced lung damage rats in EA group and EA + CCl_4 group compared to CCl_4 group and displayed its anti-inflammatory effect. A similar observation supporting for EA modulating the cytokines have already been reported in mice [27, 45]. These results indicate that inhibition of NF-kB levels by EA modulates inflammatory processes through inhibition of inflammatory cytokines such as TNF-α. Therefore, our data indicates that ellagic acid has protective effects on CCl_4-induced lung damage in rats.

It has been found that malondialdehyde, the final product of lipid peroxidation, is the major cause of cell membrane injury and used as a marker of increased oxidative stress [20, 115]. It has been reported that ellagic acid inhibited lipid peroxidation in induced V79-4 cells [25], PC12 cells,

[127], liver and heart tissue of rats [128] and in the lung of rats [20]. Studies also documented that EA efficiently reduced lipid peroxidation caused by benzidine in a time dependent manner [129]. The most recent animal study proved that EA significantly decreased MDA in serum content [24] and in liver tissue [18]. Chao et al. [27] reported on effect of ellagic acid on significantly lowering cardiac levels of Malondialdehyde and the hypo-lipidemic effects of ellagic acid were better than caffeic acid in diabetic mice. They also reported that EA decreased the levels of ROS, TNF-α and enhanced antioxidant defense via increasing GSH retention and recovering antioxidant status. Similarly, Khanduja et al. was also reported on the inhibitory effects of ellagic acid and caffeic acid on lipid peroxidation in normal peripheral blood mononuclear cells (PBMCs) [130]. In another study, Pari and Sivasankari [31] reported that treatment with EA for 21 days had efficiently protected liver tissue against the cyclosporine A-induced oxidative stress. The authors showed that administering EA to cisplatin-treated rats significantly normalized the levels of MDA, GSH, SOD and CAT, compared to those in the cisplatin-treated group. In this study, we observed a significant ($p < 0.05$) increase in MDA levels in the CCl_4 treated group as compared to those in control group and EA group. In addition, we detected that the administration of EA significantly ($p < 0.05$) reduced the lung MDA levels in EA group and EA + CCl_4 group compared to CCl_4 group. These results indicate that EA is a strong antioxidant in reducing the rate of lipid peroxidation, which could be related to decrease in the cell membrane damage in the lung.

Apoptosis of the inflammatory cells regularly results in their highly efficient clearance and normal resolution of inflammation in the lung [60]. Apoptosis, which is a controlled mechanism for physiological cell death, several proteins plays essential role in this process including, caspase-3, a significant biomarker of the cell's entry point into the execution stage of apoptosis and Bcl-2, an anti-apoptotic protein [55]. Endothelial cell necrosis appears to be an early step in the lung injury cascade, leading to lung inflammation. It was also found that a distinctive feature of apoptosis is that it occurs without causing an inflammatory reaction. thus, appearance of apoptosis in injured lungs could be part of a defense strategy to remove damaged cells from the lungs, thereby reduces the degree of injury or inflammation, as has been documented during resolution of acute respiratory distress syndrome [131-133]. Experimental animal study showed that Ellagic acid induced apoptosis through induction of caspase-3 protein levels and decreasing Bcl-2 protein levels [18]. Ellagic acid exhibits apoptosis inducting activity in (HOS cells) cancer cell line, through the activation of caspase-3 and down-regulation of Bcl-2/Bax [25]. Srigopalram et al. [134] reported a reduction in the expression of caspase-3 in experimentally-induced liver cancer in rats, whereas ellagic acid significantly increased caspase-3 protein expression in the ellagic acid-treated group when compared to the cancer bearing animals of the group. Edderkaoui et al. [33] reported that EA induced apoptosis in human pancreatic adenocarcinoma cell lines. The authors observed that EA accomplish these effects through dose-dependently decreasing NF-κB binding activity, thereby

blocking a key upstream pro-survival mechanism and activating the mitochondrial death pathway, without directly effecting mitochondria. In another study, Kasahara et al. [135] showed that inhibition of executioner caspases prevents the septal cell apoptosis and emphysema development in experimental models of emphysema. However, inhibiting caspase effects may allow damaged cells undergo non-apoptotic cell death and removal (necrotic processes), which have negative biological consequences. In this study, we observed a statistically significant ($p < 0.05$) decrease in caspase-3, an indicator of apoptosis and statistically significant increase ($p < 0.05$) in Bcl-2, an anti-apoptotic protein in the CCl_4 group as compared to the EA and EA+CCl_4 group. In addition, we detected significantly ($p < 0.05$) increased caspase-3 and decreased Bcl-2 in Ellagic acid treated groups EA and EA+CCl_4 group, which could be related to its apoptotic induction potential. These findings indicate that EA activate apoptotic pathways through upregulation of caspase-3 and downregulation of Bcl-2 in CCl_4-induced lung damage.

Excess ROS in the lung results in oxidative stress, which can alter the balance between the gene expression of proinflammatory mediators and antioxidant enzymes in favor of inflammatory mediators in the lung [11]. In response to oxidative stress, Nrf-2, a ubiquitous major transcription factor, activate cellular rescue pathways through up-regulation of antioxidant response element-bearing genes, which is encoding antioxidant defense system and cytoprotective proteins. Activation of Nrf-2 has been shown to be protective against several lung diseases that involve oxidative lung injury, inflammation and apoptosis [11, 136, 137]. Kim et al. [30] studied the effect of EA against paraquat-induced cytotoxicity in human lung carcinoma A549 cells. The authors reported the antioxidant and cytoprotective effect of ellagic acid through increasing the levels of expression and activation of Nrf-2 and its target cytoprotective and antioxidant genes. In addition, EA decreased the levels of lipid peroxidation marker, MDA, reduced the intracellular ROS level and alleviated total GSH level in A549 cells. In another study, Hseu et al. [138] studied the protective effect of EA in the UV-A-induced oxidative stress in human keratinocytes cells. The authors reported that ellagic acid activated the antioxidant defense system through the upregulation and stabilization of Nrf-2 cells. In our study, ellagic acid elevated the Nrf-2 protein expression levels in EA group compared with CCl_4 group. These results suggest that ellagic acid activate cellular rescue pathway in CCl_4-induced lung damage by enhancing the cellular antioxidative defense system.

The lungs are protected by several endogenous enzymatic and non-enzymatic antioxidants such as, catalase and glutathione, respectively. These antioxidants protect the lungs from harmful effects of a wide range of oxidants/ROS [139, 140]. Catalase represents one of the fastest and effective antioxidants in nature. It rapidly decomposes hydrogen peroxide to water and oxygen in a rate 2.5-5 x 10^6 mol/min [141]. It is regarded as the most important antioxidant enzyme in rat type II pneumocytes that breakdown exogenous hydrogen peroxide to less dangerous substances

[142]. The experiments on the effects of cigarette smoke (CS) on catalase enzyme activity demonstrated that bronchiolar epithelial catalase declines in the lungs of smokers with mild COPD. The same study also showed that the effects of CS on bronchiolar catalase enzyme expression are time-dependent in mice, rising soon after initial exposure but declining with prolonged exposure and remaining low even long after exposure to smoke has ended. The depletion of catalase in C22 cells also increased the susceptibility to CS-induced cell death, indicating a crucial role of catalase in bronchiolar epithelial cells against CS- induced cell damage [143]. These results support a fact that some nonreversible gene expression changes occur in the airway epithelium exposed to CS. Some permanent modifications in gene expression, such as catalase enzyme, might be associated with disease progression even after termination of smoking and high risk of lung cancer development in COPD patients [143, 144]. Studies on the free radical scavenging activity of EA showed that EA significantly scavenged free radicals through catalase and other antioxidant enzymes in H2O2-exposed V79 - 4 cells [25] and in liver and heart tissue of rats [18, 128]. Sudheer et al. [29] investigated the ameliorative effects of ellagic acid and N-acetylcysteine against nicotine-induced toxicity in rat peripheral blood lymphocytes. They showed that EA treatment effectively restored the Antioxidant status and catalase enzyme, decreased the lipid peroxidative indices and DNA damage. In addition, EA protection against toxicity of nicotine was as effective as N-acetylcysteine. The authors suggests the beneficial use of ellagic acid as a modifier of nicotine-induced genotoxicity. Khanduja et al. [26] investigated the Effects of administration of ellagic acid on antioxidant defense and lipid peroxidation in the liver and lungs in mice. The authors reported that oral ingestion of ellagic acid for eight weeks caused concentration dependent reduction in the MDA formation and a significant increase in the GSH levels, no changes observed in the activities of CAT and SOD in both organs. In the present findings, we observed that ellagic acid were significantly ($p < 0.05$) increased the activity of catalase enzyme in EA and CCl_4 + EA groups compared to CCl_4 group. These results suggest that ellagic acid is a potent antioxidant against CCl_4-induced lung damage through increasing the activity/or expression of antioxidant enzymes.

GSH, which is the most important tripeptide (L-glutamate, L-cysteine and glycine) intra and extracellular antioxidant synthesized in cells, plays significant roles in the regulation of cellular redox homeostasis. It is present in the epithelial lining fluid and tissue of the lung, which is high concentration of GSH is secreted by epithelial cells of the lung, where it detoxify inhaled oxidants and free radicals produced endogenously, thereby reducing lung injury to critical biological targets [39, 93, 145-148]. The GSH status of a cell suggested that might be turn out to be the most accurate single indicator of the health of cells. GSH deficiencies have been noted in a variety of respiratory diseases, including ARDS, asthma, COPD, idiopathic pulmonary fibrosis and neonatal lung damage [145, 149]. Cigarette smoke extract decreased the levels of GSH in (A549) cell lines,

effect that were involved with enhanced production of free oxygen radicals [150]. Animal study has shown that smoking evokes a strong response in the lung and can maintain at a high level for longer periods of time; this may reflect the potential value of GSH as an antioxidant that protects the lung tissue and refers to GSH adaptive response [145, 151]. Studies also reported that EA could clean up oxidative stress via lowering the formation of MDA and ROS; and improve antioxidant defense by increasing GSH retention and maintaining antioxidant enzyme activity including catalase and upregulating antioxidant enzyme expression [26-28]. Khan et al. [28] studied the protective effects of ellagic acid on lung toxicity in rats treated with cyclophosphamide and bleomycin. The authors showed that treatment with EA attenuated a marked decline in glutathione content and activities of antioxidant enzymes such as CAT and SOD decreased due to bleomycin and cyclophosphamide administration. Thus, EA prevent lungs from harmful effect of pulmonary toxicity of anticancer drugs. In the present study, glutathione levels significantly ($p < 0.05$) decreased in the CCl_4 treated group as compared to those in control group. In addition, we observed that EA administration significantly ($p < 0.05$) increased glutathione levels, which could be related to its antioxidant and free radical scavenging potential. These results indicate that ellagic acid protecting lung cells/tissues from oxidative damage through activating Nrf-2, thereby enhancing cellular antioxidant potential and inducing the expression levels of GSH and catalase enzyme activity. Thus, EA act as an effective antioxidant, minimizing the CCl_4-induced damage in the lung.

5. CONCLUSION

In conclusion, the results of this study establishes that ellagic acid, a polyphenolic compound, is able to ameliorate the lung damage in rats via several regulatory pathways. These include the intervention of NF-kB pathway, thereby subsequently reducing the inflammatory markers, COX-2 and TNF-α; activation of antioxidant response element-bearing- genes using the Nrf-2; enhance the activity of antioxidants and antioxidant enzymes, GSH and catalase enzyme, respectively; modulation of apoptosis via inducing caspase-3 protein expression and inhibition of Bcl-2 protein expression; and decreasing MDA, final product of lipid peroxidation. Interestingly, in the present results, no statistically significant difference ($p > 0.05$) were observed between EA group and control group for GSH, CAT, MDA, Bcl-2, NF-kB, TNF-α and Nrf-2, EA only attenuated the altered levels of these markers, which may be ensuring no negative reactions happened with the use of EA. Thus, EA showed its protective role in lung damage through suppressing genes responsible for the specific inflammation and disturbance of biochemical systems, activating cellular rescue pathways and apoptosis-inducing activity, as illustrated in Figure 5.1. Our results establish the therapeutic role of EA in the treatment of lung damage and that in the future this may have the potential to be used as a medication for the prevention or attenuation of lung diseases.

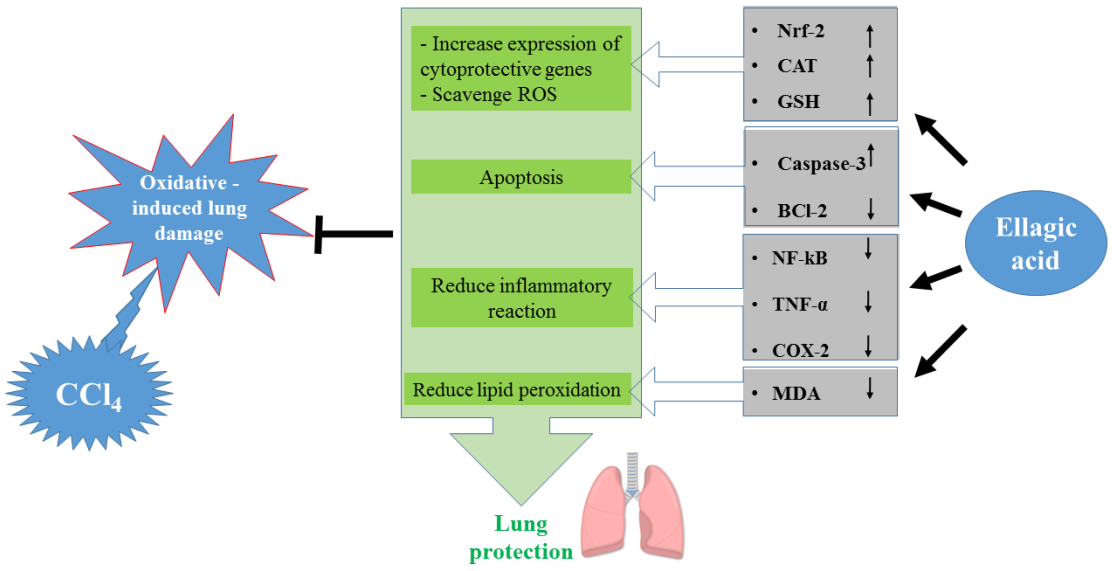

Figure 5.1. Ameliorative effects of EA against CCl_4-induced lung damage through increasing expression of cytoprotective genes, antioxidant and ROS scavenging activities, apoptosis- inducing activity, reducing inflammatory reaction and reducing lipid peroxidation.

RECOMMENDATIONS

Further studies should be designed to investigate the bioavailability, metabolism and multiple potential of EA and their derived metabolite in human subjects. Although, it is very difficult to determine the minimal dose for biological effects of polyphenols in humans, studies on the concentration of polyphenols and biological effects would be of interest.

REFERENCES

[1] Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017). Oxidative stress: harms and benefits for human health, *Oxidative Medicine and Cellular Longevity*, 2017, 13. doi: 10.1155/2017/8416763.

[2] Lee, I.T. and Yang, C.M. (2012). Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases, *Biochemical Pharmacological*, 84, 581-90. doi: 10.1016/j.bcp.2012.05.005.

[3] Park, H.S., Kim, S.R., and Lee, Y.C. (2009). Impact of oxidative stress on lung diseases, *Respirology*, 14, 27-38. doi: 10.1111/j.1440-1843.2008.01447.x.

[4] Rogers, L.K. and Cismowski, M.J. (2018). Oxidative stress in the lung – The essential paradox, *Current Opinion in Toxicology*, 7, 37-43. doi: 10.1016/j.cotox.2017.09.001.

[5] Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: a cancer journal for clinicians*, 68, 394-424. doi: 10.3322/caac.21492.

[6] Mathers, C.D. and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030, *PLoS medicine*, 3, e442. doi: 10.1371/journal.pmed.0030442.

[7] Fridovich, I. and Freeman, B. (1986). Antioxidant defenses in the lung, *Annual review of physiology*, 48, 693-702. doi: 10.1146/annurev.ph.48.030186.003401.

[8] Weber, L.W., Boll, M., and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, *Critical reviews in toxicology*, 33, 105-136. doi: 10.1080/713611034.

[9] Bargagli, E., Olivieri, C., Bennett, D., Prasse, A., Muller-Quernheim, J., and Rottoli, P. (2009). Oxidative stress in the pathogenesis of diffuse lung diseases: a review, *Respiratory medicine*, 103, 1245-1256. doi: 10.1016/j.rmed.2009.04.014.

[10] Gutteridge, J.M.C. (1995). Lipid-Peroxidation and Antioxidants as Biomarkers of Tissue-Damage, *Clinical chemistry*, 41, 1819-1828.

[11] Valavanidis, A., Vlachogianni, T., Fiotakis, K., and Loridas, S. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms, *International journal of environmental research and public health*, 10, 3886-3907. doi: 10.3390/ijerph10093886.

[12] Makni, M., Chtourou, Y., Barkallah, M., and Fetoui, H. (2012). Protective effect of vanillin against carbon tetrachloride (CCl4)-induced oxidative brain injury in rats, *Toxicology and industrial health*, 28, 655-662. doi: 10.1177/0748233711420472.

[13] Ahmad, F., Cowan, D., and Sun, A. (1987). Detection of free radical formation in various tissues after acute carbon tetrachloride administration in gerbil, *Life sciences*, 41, 2469-2475. doi: 10.1016/0024-3205(87)90673-4.

[14] Khan, R.A., Alkreathy, H.M., Saboorshah, A., Ahmed, M., and Khan, S. (2016). Protective effects of Trifolium alexandrinum L. against lung injury induced by environmental toxin CCl4 in experimental rats, *Food & nutrition research*, 60, 30433. doi: 10.3402/fnr.v60.30433.

[15] Ritesh, K., Suganya, A., Dileepkumar, H., Rajashekar, Y., and Shivanandappa, T. (2015). A single acute hepatotoxic dose of CCl4 causes oxidative stress in the rat brain, *Toxicology reports*, 2, 891-895. doi: 10.1016/j.toxrep.2015.05.012.

[16] Ganie, S.A., Haq, E., Hamid, A., Qurishi, Y., Mahmood, Z., Zargar, B.A., Masood, A., and Zargar, M.A. (2011). Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of Podophyllum hexandrum, *BMC complementary and alternative medicine*, 11, 17. doi: 10.1186/1472-6882-11-17.

[17] Khan, R.A., Khan, M.R., and Sahreen, S. (2012). Protective effect of Sonchus asper extracts against experimentally induced lung injuries in rats: a novel study, *Experimental and toxicologic pathology*, 64, 725-731. doi: 10.1016/j.etp.2011.01.007.

[18] Aslan, A., Gok, O., Erman, O., and Kuloglu, T. (2018). Ellagic acid impedes carbontetrachloride-induced liver damage in rats through suppression of NF-kB, Bcl-2 and regulating Nrf-2 and caspase pathway, *Biomedicine & Pharmacotherapy*, 105, 662-669. doi: 10.1016/j.biopha.2018.06.020.

[19] de Freitas Alves, C., Angeli, G.N., Favarin, D.C., Lemos de Andrade, E., Lazo Chica, J.E., Faccioli, L.H., Roberto da Silva, P., and de Paula Rogerio, A. (2013). The effects of proresolution of ellagic acid in an experimental model of allergic airway inflammation, *Mediators of inflammation*, 2013, 9. doi: 10.1155/2013/863198.

[20] Gul', M., Aliosmanoglu', I., Uslukaya', O., Firat, U., Yüksel, H., Gümüs', M., and Ulger, B. (2013). The protective effect of ellagic acid on lung damage caused by experimental obstructive jaundice model, *Acta Chirurgica Belgica*, 113, 285-289. doi: Doi 10.1080/00015458.2013.11680929.

[21] Priyadarsini, K.I., Khopde, S.M., Kumar, S.S., and Mohan, H. (2002). Free radical studies of ellagic acid, a natural phenolic antioxidant, *Journal of agricultural and food chemistry*, 50, 2200-2206. doi: 10.1021/jf011275g.

[22] Vattem, D. and Shetty, K. (2005). Biological functionality of ellagic acid: a review, *Journal of food biochemistry*, 29, 234-266. doi: 10.1111/j.1745-4514.2005.00031.x.

[23] Umesalma, S., Nagendraprabhu, P., and Sudhandiran, G. (2015). Ellagic acid inhibits proliferation and induced apoptosis via the Akt signaling pathway in HCT-15 colon adenocarcinoma cells, *Molecular and cellular biochemistry*, 399, 303-313. doi: 10.1007/s11010-014-2257-2.

[24] Zhou, B., Li, Q., Wang, J., Chen, P., and Jiang, S. (2019). Ellagic acid attenuates streptozocin induced diabetic nephropathy via the regulation of oxidative stress and inflammatory signaling, *Food and Chemical Toxicology*, 123, 16-27. doi: 10.1016/j.fct.2018.10.036.

[25] Han, D.H., Lee, M.J., and Kim, J.H. (2006). Antioxidant and apoptosis-inducing activities of ellagic acid, *Anticancer research*, 26, 3601-3606.

[26] Majid, S., Khanduja, K.L., Gandhi, R.K., Kapur, S., and Sharma, R.R. (1991). Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice, *Biochemical Pharmacology*, 42, 1441-1445. doi: 10.1016/0006-2952(91)90457-g.

[27] Chao, P.-c., Hsu, C.-c., and Yin, M.-c. (2009). Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice, *Nutrition & metabolism*, 6, 33. doi: 10.1186/1743-7075-6-33.

[28] Saba, Khan, S., Parvez, S., Chaudhari, B., Ahmad, F., Anjum, S., and Raisuddin, S. (2013). Ellagic acid attenuates bleomycin and cyclophosphamide-induced pulmonary toxicity in Wistar rats, *Food and Chemical Toxicology*, 58, 210-219. doi: 10.1016/j.fct.2013.03.046.

[29] Sudheer, A.R., Muthukumaran, S., Devipriya, N., and Menon, V.P. (2007). Ellagic acid, a natural polyphenol protects rat peripheral blood lymphocytes against nicotine-induced cellular and DNA damage in vitro: with the comparison of N-acetylcysteine, *Toxicology*, 230, 11-21. doi: 10.1016/j.tox.2006.10.010.

[30] Kim, Y.-S., Zerin, T., and Song, H.-Y. (2013). Antioxidant action of ellagic acid ameliorates paraquat-induced A549 cytotoxicity, *Biological and Pharmaceutical Bulletin*, 36, 609-615. doi: 10.1248/bpb.b12-00990.

[31] Pari, L. and Sivasankari, R. (2008). Effect of ellagic acid on cyclosporine A-induced oxidative damage in the liver of rats, *Fundamental & Clinical Pharmacology*, 22, 395-401. doi: 10.1111/j.1472-8206.2008.00609.x.

[32] El-Shitany, N.A., El-Bastawissy, E.A., and El-desoky, K. (2014). Ellagic acid protects against carrageenan-induced acute inflammation through inhibition of nuclear factor kappa B, inducible cyclooxygenase and proinflammatory cytokines and enhancement of interleukin-10 via an antioxidant mechanism, *International immunopharmacology*, 19, 290-299. doi: 10.1016/j.intimp.2014.02.004.

[33] Edderkaoui, M., Odinokova, I., Ohno, I., Gukovsky, I., Go, V.L.W., Pandol, S.J., and Gukovskaya, A.S. (2008). Ellagic acid induces apoptosis through inhibition of nuclear factor kappa B in pancreatic cancer cells, *World journal of gastroenterology*, 14, 3672-3680. doi: 10.3748/wjg.14.3672.

[34] Shier, D., Butler, J., Lewis, R., and McGraw-Hill Higher, E., (2010). *Hole's Human anatomy & physiology*, McGraw-Hill Higher Education, New York, N.Y.

[35] Mader, S.S., (2008). *Mader's Understanding Human Anatomy & Physiology*, McGraw-Hill Higher Education,

[36] Eroschenko, V.P. and Di Fiore, M.S., (2013). *DiFiore's atlas of histology with functional correlations*, Lippincott Williams & Wilkins,

[37] Kingma, P. and Jobe, A.H., *5 - The Surfactant System*, in *Kendig's Disorders of the Respiratory Tract in Children*, R.W. Wilmott, R. Deterding, A. Li, F. Ratjen, P. Sly, H.J. Zar, and A. Bush, Editors. 2019, Elsevier: Philadelphia. p. 57-62. e2.

[38] Iyawe, V.I. and Omorogiuwa, A. (2018). The Respiratory System Has a Resourceful Defense Organization; A Review, *Journal of Applied Sciences and Environmental Management*, 22, 1871–1876. doi: 10.4314/jasem.v22i11.24.

[39] Yadav, A.K., Bracher, A., Doran, S.F., Leustik, M., Squadrito, G.L., Postlethwait, E.M., and Matalon, S. (2010). Mechanisms and modification of chlorine-induced lung injury in animals, *Proceedings of the American Thoracic Society*, 7, 278-283. doi: 10.1513/pats.201001-009SM.

[40] Zeb, A. (2018). Ellagic acid in suppressing in vivo and in vitro oxidative stresses, *Molecular and cellular biochemistry*, 448, 27-41. doi: 10.1007/s11010-018-3310-3.

[41] Zhang, H.-M., Zhao, L., Li, H., Xu, H., Chen, W.-W., and Tao, L. (2014). Research progress on the anticarcinogenic actions and mechanisms of ellagic acid, *Cancer biology & medicine*, 11, 92. doi: 10.7497/j.issn.2095-3941.2014.02.004.

[42] Girish, C. and Pradhan, S.C., *Chapter 44 - Herbal Drugs on the Liver*, in *Liver Pathophysiology*, P. Muriel, Editor 2017, Academic Press: Boston. p. 605-620.

[43] Shakeri, A., Zirak, M.R., and Sahebkar, A. (2018). Ellagic Acid: A Logical Lead for Drug Development?, *Current pharmaceutical design*, 24, 106-122. doi: 10.2174/1381612823666171115094557.

[44] Landete, J.M. (2011). Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health, *Food Research International*, 44, 1150-1160. doi: 10.1016/j.foodres.2011.04.027.

[45] Cornélio Favarin, D., Martins Teixeira, M., Lemos de Andrade, E., de Freitas Alves, C., Lazo Chica, J.E., Artério Sorgi, C., Faccioli, L.H., and Paula Rogerio, A. (2013). Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice, *Mediators of inflammation*, 2013, 13. doi: 10.1155/2013/164202.

[46] Smart, R.C., Huang, M.-T., Chang, R.L., Sayer, J.M., Jerina, D.M., and Conney, A.H. (1986). Disposition of the naturally occurring antimutagenic plant phenol, ellagic acid, and its synthetic derivatives, 3-O-decylellagic acid and 3, 3'-di-O-methylellagic acid in mice, *Carcinogenesis*, 7, 1663-1667. doi: 10.1093/carcin/7.10.1663.

[47] Seeram, N.P., Henning, S.M., Zhang, Y., Suchard, M., Li, Z., and Heber, D. (2006). Pomegranate Juice Ellagitannin Metabolites Are Present in Human Plasma and Some Persist in Urine for Up to 48 Hours, *The Journal of Nutrition*, 136, 2481-2485. doi: 10.1093/jn/136.10.2481.

[48] Mertens-Talcott, S.U., Jilma-Stohlawetz, P., Rios, J., Hingorani, L., and Derendorf, H. (2006). Absorption, metabolism, and antioxidant effects of pomegranate (Punica granatum L.) polyphenols after ingestion of a standardized extract in healthy human volunteers, *Journal of agricultural and food chemistry*, 54, 8956-8961. doi: 10.1021/jf061674h.

[49] Seeram, N.P., Lee, R., and Heber, D. (2004). Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (Punica granatum L.) juice, *Clinica Chimica Acta*, 348, 63-68. doi: 10.1016/j.cccn.2004.04.029.

[50] Seeram, N.P., Zhang, Y., McKeever, R., Henning, S.M., Lee, R.-p., Suchard, M.A., Li, Z., Chen, S., Thames, G., and Zerlin, A. (2008). Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects, *Journal of medicinal food*, 11, 390-394. doi: 10.1089/jmf.2007.650.

[51] Kawabata, K., Yoshioka, Y., and Terao, J. (2019). Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols, *Molecules*, 24, 370. doi: 10.3390/molecules24020370.

[52] González-Sarrías, A., García-Villalba, R., Núñez-Sánchez, M.Á., Tomé-Carneiro, J., Zafrilla, P., Mulero, J., Tomás-Barberán, F.A., and Espín, J.C. (2015). Identifying the limits for ellagic acid bioavailability: A crossover pharmacokinetic study in healthy volunteers after consumption of pomegranate extracts, *Journal of Functional Foods*, 19, 225-235. doi: 10.1016/j.jff.2015.09.019.

[53] Ayhanci, A., Cengiz, M., Kutlu, H.M., and Vejselova, D. (2016). Protective effects of ellagic acid in D-galactosamine-induced kidney damage in rats, *Cytotechnology*, 68, 1763-1770. doi: 10.1007/s10616-015-9928-z.

[54] Hassoun, E., Walter, A., Alsharif, N.Z., and Stohs, S. (1997). Modulation of TCDD-induced fetotoxicity and oxidative stress in embryonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid, *Toxicology*, 124, 27-37. doi: 10.1016/s0300-483x(97)00127-3.

[55] Nagata, S. (2018). Apoptosis and clearance of apoptotic cells, *Annual review of immunology*, 36, 489-517. doi: 10.1146/annurev-immunol-042617-053010.

[56] Chandar, N. and Viselli, S., (2012). *Cell and molecular biology*, Lippincott Williams & Wilkins,

[57] Choudhury, J.D., Kumar, S., Mayank, V., Mehta, J., and Bardalai, D. (2012). A review on apoptosis and its different pathway, *International Journal of Biological and Pharmaceutical Research*, 3, 848-861.

[58] Sauler, M., Bazan, I.S., and Lee, P.J. (2019). Cell Death in the Lung: The Apoptosis–Necroptosis Axis, *Annual review of physiology*, 81, 375-402.

[59] Lu, Q., Harrington, E.O., and Rounds, S. (2005). Apoptosis and lung injury, *The Keio journal of medicine*, 54, 184-189. doi: 10.2302/kjm.54.184.

[60] Henson, P.M. and Tuder, R.M. (2008). Apoptosis in the lung: induction, clearance and detection, *Am J Physiol Lung Cell Mol Physiol*, 294, L601-11. doi: 10.1152/ajplung.00320.2007.

[61] Takata, T., Tanaka, F., Yamada, T., Yanagihara, K., Otake, Y., Kawano, Y., Nakagawa, T., Miyahara, R., Oyanagi, H., and Inui, K. (2001). Clinical significance of caspase-3 expression in pathologic-stage I, nonsmall-cell lung cancer, *International journal of cancer*, 96, 54-60. doi: 10.1002/ijc.10347.

[62] Biosciences, B. (2012). Caspase-3 activation-an indicator of apoptosis in image-based assays, *Appl Note*, 1-4.

[63] Abu-Qare, A.W. and Abou-Donia, M.B. (2001). Biomarkers of apoptosis: release of cytochrome c, activation of caspase-3, induction of 8-hydroxy-2'-deoxyguanosine, increased 3-nitrotyrosine, and alteration of p53 gene, *Journal of toxicology and environmental health. Part B, Critical reviews*, 4, 313-332. doi: 10.1080/109374001301419737.

[64] Youle, R.J. and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death, *Nature reviews Molecular cell biology*, 9, 47-59. doi: 10.1038/nrm2308.

[65] Czabotar, P.E., Lessene, G., Strasser, A., and Adams, J.M. (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy, *Nature reviews Molecular cell biology*, 15, 49. doi: 10.1038/nrm3722.

[66] Cakir, E., Yilmaz, A., Demirag, F., Oguztuzun, S., Sahin, S., Yazici, U.E., and Aydin, M. (2011). Prognostic significance of micropapillary pattern in lung adenocarcinoma and expression of apoptosis-related markers: caspase-3, bcl-2, and p53, *Apmis*, 119, 574-580. doi: 10.1111/j.1600-0463.2011.02778.x.

[67] Gilmore, T.D. (2006). Introduction to NF-κB: players, pathways, perspectives, *Oncogene*, 25, 6680-6684. doi: 10.1038/sj.onc.1209954.

[68] Li, N. and KARIN, M. (1999). Is NF-κB the sensor of oxidative stress?, *The FASEB journal*, 13, 1137-1143.

[69] Brigelius-Flohé, R. and Flohe, L. (2011). Basic Principles and Emerging Concepts in the Redox Control of Transcription Factors, *Antioxidants & redox signaling*, 15, 2335-81. doi: 10.1089/ars.2010.3534.

[70] Chen, W., Li, Z., Bai, L., and Lin, Y. (2011). NF-kappaB in lung cancer, a carcinogenesis mediator and a prevention and therapy target. Frontiers in bioscience (Landmark edition), 16, 1172-1185. doi: 10.2741/3782.

[71] Wladis, E.J., Lau, K.W., and Adam, A.P. (2019). Nuclear Factor Kappa-B is Enriched in Eyelid Specimens of Rosacea: Implications for Pathogenesis and Therapy, *American journal of ophthalmology*, 201, 72-81. doi: 10.1016/j.ajo.2019.01.018.

[72] Serasanambati, M. and Chilakapati, S.R. (2016). Function of nuclear factor kappa B (NF-kB) in human diseases-a review, *South Indian Journal of Biological Sciences*, 2, 368-387.

[73] Wang, J., Liu, Y.-T., Xiao, L., Zhu, L., Wang, Q., and Yan, T. (2014). Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway, *Inflammation*, 37, 2085-2090. doi: 10.1007/s10753-014-9942-x.

[74] Umesalma, S. and Sudhandiran, G. (2010). Differential inhibitory effects of the polyphenol ellagic acid on inflammatory mediators NF-κB, iNOS, COX-2, TNF-α, and IL-6 in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis, *Basic & clinical pharmacology & toxicology*, 107, 650-655. doi: 10.1111/j.1742-7843.2010.00565.x.

[75] Goto, T., Ishizaka, A., Kobayashi, F., Kohno, M., Sawafuji, M., Tasaka, S., Ikeda, E., Okada, Y., Maruyama, I., and Kobayashi, K. (2004). Importance of tumor necrosis factor-α cleavage process in post-transplantation lung injury in rats, *American journal of respiratory and critical care medicine*, 170, 1239-1246. doi: 10.1164/rccm.200402-146OC.

[76] Mukhopadhyay, S., Hoidal, J.R., and Mukherjee, T.K. (2006). Role of TNFα in pulmonary pathophysiology, *Respiratory research*, 7, 125. doi: 10.1186/1465-9921-7-125.

[77] Wu, Y.-d. and Zhou, B. (2010). TNF-α/NF-κB/Snail pathway in cancer cell migration and invasion, *British journal of cancer*, 102, 639. doi: 10.1038/sj.bjc.6605530.

[78] Casale, T.B., Costa, J.J., and Galli, S.J. (1996). TNF alpha is important in human lung allergic reactions, *American journal of respiratory cell and molecular biology*, 15, 35-44. doi: DOI 10.1165/ajrcmb.15.1.8679220.

[79] Sobolewski, C., Cerella, C., Dicato, M., Ghibelli, L., and Diederich, M. (2010). The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies, *International journal of cell biology*, 2010, 215158. doi: 10.1155/2010/215158.

[80] Cao, Y. and Prescott, S.M. (2002). Many actions of cyclooxygenase-2 in cellular dynamics and in cancer, *Journal of cellular physiology*, 190, 279-286. doi: 10.1002/jcp.10068.

[81] Liu, C.H., Chang, S.-H., Narko, K., Trifan, O.C., Wu, M.-T., Smith, E., Haudenschild, C., Lane, T.F., and Hla, T. (2001). Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice, *Journal of Biological Chemistry*, 276, 18563-18569. doi: DOI 10.1074/jbc.M010787200.

[82] Dannenberg, A.J., Altorki, N.K., Boyle, J.O., Dang, C., Howe, L.R., Weksler, B.B., and Subbaramaiah, K. (2001). Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer, *The Lancet Oncology*, 2, 544-551. doi: https://doi.org/10.1016/S1470-2045(01)00488-0.

[83] Crosby, C.G. and DuBois, R.N., *The cyclooxygenase-2 pathway as a target for treatment or prevention of cancer*, 2003, Taylor & Francis. p. 1-7. doi: 10.1517/14728214.8.1.1.

[84] Gandhi, J., Khera, L., Gaur, N., Paul, C., and Kaul, R. (2017). Role of Modulator of Inflammation Cyclooxygenase-2 in Gammaherpesvirus Mediated Tumorigenesis, *Frontiers in Microbiology*, 8, 538. doi: 10.3389/fmicb.2017.00538.

[85] Cho, H.-Y., Reddy, S.P., and Kleeberger, S.R. (2006). Nrf2 defends the lung from oxidative stress, *Antioxidants & redox signaling*, 8, 76-87. doi: 10.1089/ars.2006.8.76.

[86] Liu, Q., Gao, Y., and Ci, X. (2019). Role of Nrf2 and Its Activators in Respiratory Diseases, *Oxidative Medicine and Cellular Longevity*, 2019, 17. doi: 10.1155/2019/7090534.

[87] Ahmed, S.M.U., Luo, L., Namani, A., Wang, X.J., and Tang, X. (2017). Nrf2 signaling pathway: pivotal roles in inflammation, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1863, 585-597. doi: 10.1016/j.bbadis.2016.11.005.

[88] Wardyn, J.D., Ponsford, A.H., and Sanderson, C.M. (2015). Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways, *Biochemical society transactions*, 43, 621-626. doi: 10.1042/BST20150014.

[89] Cho, H.-Y., Jedlicka, A.E., Reddy, S.P.M., Kensler, T.W., Yamamoto, M., Zhang, L.-Y., and Kleeberger, S.R. (2002). Role of NRF2 in Protection Against Hyperoxic Lung Injury in Mice, *American journal of respiratory cell and molecular biology*, 26, 175-182. doi: 10.1165/ajrcmb.26.2.4501.

[90] Ighodaro, O. and Akinloye, O. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid, *Alexandria Journal of Medicine*, 54, 287-293. doi: 10.1016/j.ajme.2017.09.001.

[91] Zamocky, M., Furtmüller, P.G., and Obinger, C. (2008). Evolution of catalases from bacteria to humans, *Antioxidants & redox signaling*, 10, 1527-1548. doi: 10.1089/ars.2008.2046.

[92] Odajima, N., Betsuyaku, T., Nagai, K., Moriyama, C., Wang, D.-H., Takigawa, T., Ogino, K., and Nishimura, M. (2010). The role of catalase in pulmonary fibrosis, *Respiratory research*, 11, 183. doi: 10.1186/1465-9921-11-183.

[93] Forman, H.J., Zhang, H., and Rinna, A. (2009). Glutathione: Overview of its protective roles, measurement, and biosynthesis, *Molecular Aspects of Medicine*, 30, 1-12. doi: https://doi.org/10.1016/j.mam.2008.08.006.

[94] Coursin, D., Cihla, H., Sempf, J., Oberley, T., and Oberley, L. (1996). An immunohistochemical analysis of antioxidant and glutathione S-transferase enzyme levels in normal and neoplastic human lung, *Histology and histopathology*.

[95] Cobanoglu, U., Demir, H., Duran, M., Şehitogullari, A., Mergan, D., and Demir, C. (2010). Erythrocyte catalase and carbonic anhydrase activities in lung cancer, *Asian Pac J Cancer Prev*, 11, 1377-82.

[96] Ghosh, S., Janocha, A.J., Aronica, M.A., Swaidani, S., Comhair, S.A., Xu, W., Zheng, L., Kaveti, S., Kinter, M., and Hazen, S.L. (2006). Nitrotyrosine proteome survey in asthma identifies oxidative mechanism of catalase inactivation, *The Journal of Immunology*, 176, 5587-5597. doi: 10.4049/jimmunol.176.9.5587.

[97] Narayanankutty, A., Jdevagiri, J., and Narayanankutty, V. (2019). Glutathione, an antioxidant tripeptide: Dual roles in Carcinogenesis and Chemoprevention, *Current protein & peptide science*, 20, 907-917. doi: 10.2174/1389203720666190206130003.

[98] Rahman, I., Biswas, S.K., Jimenez, L.A., Torres, M., and Forman, H. (2005). Glutathione, stress responses, and redox signaling in lung inflammation, *Antioxidants & redox signaling*, 7, 42-59. doi: 10.1089/ars.2005.7.42.

[99] Biswas, S.K. and Rahman, I. (2009). Environmental toxicity, redox signaling and lung inflammation: The role of glutathione, *Molecular Aspects of Medicine*, 30, 60-76. doi: https://doi.org/10.1016/j.mam.2008.07.001.

[100] Valenzuela, A. (1991). The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress, *Life sciences*, 48, 301-309. doi: 10.1016/0024-3205(91)90550-u.

[101] VARŠKEVIČIENÉ, Z., ČERNIAUSKIENÉ, R., and Grybauskas, P. (1984). Effect of a-Tocopherol on the Production of Malondialdehyde in Rat Tissue Homogenates after Hypobaric Exposure, *Gen. Physiol. Biophys*, 3, 47-53.

[102] Khan, H.A., Abdelhalim, M.A.K., Al-Ayed, M.S., and Alhomida, A.S. (2012). Effect of gold nanoparticles on glutathione and malondialdehyde levels in liver, lung and heart of rats, *Saudi journal of biological sciences*, 19, 461-464. doi: 10.1016/j.sjbs.2012.06.005.

[103] Turgut, G., Enli, Y., Kaptanoglu, B., Turgut, S., and Genç, O. (2006). Changes in the levels of MDA and GSH in mice serum, liver and spleen after aluminum administration, *East J Med*, 11, 7-12.

[104] Aslan, A., Boydak, D., Can, M.I., and Kuloglu, T. (2015). Nigella sativa improves the carbon tetrachloride-induced lung damage in rats through repression of erk/akt pathway, *Bangladesh Journal of Pharmacology*, 10, 654-659. doi: 10.3329/bjp.v10i3.23486.

[105] Lu, K.-H., Weng, C.-Y., Chen, W.-C., and Sheen, L.-Y. (2017). Ginseng essence, a medicinal and edible herbal formulation, ameliorates carbon tetrachloride-induced oxidative stress and liver injury in rats, *Journal of ginseng research*, 41, 316-325. doi: 10.1016/j.jgr.2016.06.002.

[106] Grossi, M.R., Berni, A., Pepe, G., Filippi, S., Meschini, R., Papeschi, C., Natarajan, A.T., and Palitti, F. (2014). Evaluation of the effects of ellagic acid (EA) on 7, 12-dimethylbenz (α) anthracene (DMBA) induced micronuclei in mammalian cells in vitro and in vivo, *Toxicology letters*, 224, 240-245. doi: 10.1016/j.toxlet.2013.10.012.

[107] Aslan, A., (2011). *The effect of lycopene on the cyclooxygenase (cox-2), caspase-3, caspase-9, bax, bcl-2, p53 Protein expression and DNA damage in rats with azoxhymethane-induced colorectal cancer*, Phd Thesis.

[108] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *nature*, 227, 680. doi: 10.1038/227680a0.

[109] Luck, H. (1963). Catalase//Methods in enzymatic analysis/H.–U. Bergmeyer ed, *Verlag Chemie, GmbH, Academic Press, New York*, 885-894.

[110] Ellman, G.L. (1959). Tissue sulfhydryl groups, *Archives of biochemistry and biophysics*, 82, 70-77. doi: 10.1016/0003-9861(59)90090-6.

[111] Yiğit, M., (2015). *Effects of astaxanthin on antioxidant parameters (GSH, GSH-Px, MDA), Ca2+ signaling and apoptosis levels in ARPE-19 cells on cigarette smoke-related hydrokuinon induced oxidative stress model*, SDÜ Tıp Fakültesi. Isparta.

[112] Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analytical biochemistry*, 95, 351-358. doi: 10.1016/0003-2697(79)90738-3.

[113] Murata, M., Thanan, R., Ma, N., and Kawanishi, S. (2012). Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis, *BioMed Research International*, 2012, 623019. doi: 10.1155/2012/623019.

[114] Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y., Oikawa, S., and Murata, M. (2016). Nitrative and oxidative DNA damage in infection-related carcinogenesis in relation to cancer stem cells, *Genes and environment*, 38, 26. doi: 10.1186/s41021-016-0055-7.

[115] Gu, L., Deng, W.-s., Liu, Y., Jiang, C.-h., Sun, L.-c., Sun, X.-f., Xu, Q., and Zhou, H. (2014). Ellagic acid protects Lipopolysaccharide/D-galactosamine-induced acute hepatic injury in mice, *International immunopharmacology*, 22, 341-345. doi: 10.1016/j.intimp.2014.07.005.

[116] Zhou, E., Fu, Y., Wei, Z., and Yang, Z. (2014). Inhibition of allergic airway inflammation through the blockage of NF-kappaB activation by ellagic acid in an ovalbumin-induced mouse asthma model, *Food Funct*, 5, 2106-12. doi: 10.1039/c4fo00384e.

[117] Krysan, K., Reckamp, K.L., Sharma, S., and Dubinett, S.M. (2006). The potential and rationale for COX-2 inhibitors in lung cancer, *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 6, 209-220. doi: 10.2174/187152006776930882.

[118] Sandler, A.B. and Dubinett, S.M. *COX-2 inhibition and lung cancer*. in *Seminars in Oncology*. 2004. Elsevier.

[119] Hashemi Goradel, N., Najafi, M., Salehi, E., Farhood, B., and Mortezaee, K. (2019). Cyclooxygenase-2 in cancer: A review, *Journal of cellular physiology*, 234, 5683-5699. doi: 10.1002/jcp.27411.

[120] Yao, R., Rioux, N., Castonguay, A., and You, M. (2000). Inhibition of COX-2 and induction of apoptosis: two determinants of nonsteroidal anti-inflammatory drugs' chemopreventive efficacies in mouse lung tumorigenesis, *Experimental lung research*, 26, 731-742. doi: 10.1080/01902140150216783.

[121] Karlsson, S., Nånberg, E., Fjaeraa, C., and Wijkander, J. (2010). Ellagic acid inhibits lipopolysaccharide-induced expression of enzymes involved in the synthesis of prostaglandin E 2 in human monocytes, *British journal of nutrition*, 103, 1102-1109. doi: 10.1017/S0007114509992935.

[122] Attilio, P., Merritt, C., Sims, J., Kane, N., and Sullivan, J.O. (2010). The effect of ellagic acid on platelet activation as measured by the quantification of P-selectin using flow cytometry, *AANA journal*, 78.

[123] Zhang, K., Gharaee-Kermani, M., McGarry, B., Remick, D., and Phan, S.H. (1997). TNF-alpha-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis, *The Journal of Immunology*, 158, 954-959.

[124] Tanigaki, R., Takahashi, R., Nguyen, M.T.T., Nguyen, N.T., Do, T.V.N., Nguyen, H.X., and Kataoka, T. (2019). 4-Hydroxypanduratin A and Isopanduratin A Inhibit Tumor Necrosis Factor α-Stimulated Gene Expression and the Nuclear Factor κB-Dependent Signaling Pathway in Human Lung Adenocarcinoma A549 Cells, *Biological and Pharmaceutical Bulletin*, 42, 26-33. doi: 10.1248/bpb.b18-00457.

[125] Azzolina, A., Bongiovanni, A., and Lampiasi, N. (2003). Substance P induces TNF-α and IL-6 production through NFκB in peritoneal mast cells, *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1643, 75-83.

[126] Zhang, X., Song, K., Xiong, H., Li, H., Chu, X., and Deng, X. (2009). Protective effect of florfenicol on acute lung injury induced by lipopolysaccharide in mice, *International immunopharmacology*, 9, 1525-1529. doi: 10.1016/j.intimp.2009.09.007.

[127] Pavlica, S. and Gebhardt, R. (2005). Protective effects of ellagic and chlorogenic acids against oxidative stress in PC12 cells, *Free Radical Research*, 39, 1377-1390. doi: 10.1080/09670260500197660.

[128] Yüce, A., Ateşşahin, A., Çeribaşı, A.O., and Aksakal, M. (2007). Ellagic acid prevents cisplatin-induced oxidative stress in liver and heart tissue of rats, *Basic & clinical pharmacology & toxicology*, 101, 345-349. doi: 10.1111/j.1742-7843.2007.00129.x.

[129] Makena, P.S. and Chung, K.-T. (2007). Effects of various plant polyphenols on bladder carcinogen benzidine-induced mutagenicity, *Food and Chemical Toxicology*, 45, 1899-1909. doi: https://doi.org/10.1016/j.fct.2007.04.007.

[130] Khanduja, K.L., Avti, P.K., Kumar, S., Mittal, N., Sohi, K.K., and Pathak, C.M. (2006). Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood mononuclear cells: a Bcl-2 independent mechanism, *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1760, 283-289. doi: 10.1016/j.bbagen.2005.12.017.

[131] Mantell, L.L., Kazzaz, J.A., Xu, J., Palaia, T.A., Piedboeuf, B., Hall, S., Rhodes, G.C., Niu, G., Fein, A.F., and Horowitz, S. (1997). Unscheduled apoptosis during acute inflammatory lung injury, *Cell death and differentiation*, 4, 600. doi: 10.1038/sj.cdd.4400278.

[132] Polunovsky, V.A., Chen, B., Henke, C., Snover, D., Wendt, C., Ingbar, D.H., and Bitterman, P.B. (1993). Role of mesenchymal cell death in lung remodeling after injury, *The Journal of clinical investigation*, 92, 388-397. doi: 10.1172/JCI116578.

[133] Uhal, B.D., Joshi, I., True, A.L., Mundle, S., Raza, A., Pardo, A., and Selman, M. (1995). Fibroblasts isolated after fibrotic lung injury induce apoptosis of alveolar epithelial cells in vitro, *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 269, L819-L828. doi: 10.1152/ajplung.1995.269.6.L819.

[134] Srigopalram, S., Ilavenil, S., and Jayraaj, I.A. (2012). Apoptosis associated inhibition of DEN-induced hepatocellular carcinogenesis by ellagic acid in experimental rats, *Biomedicine & Preventive Nutrition*, 2, 1-8. doi: 10.1016/j.bionut.2011.12.003.

[135] Kasahara, Y., Tuder, R.M., Taraseviciene-Stewart, L., Le Cras, T.D., Abman, S., Hirth, P.K., Waltenberger, J., and Voelkel, N.F. (2000). Inhibition of VEGF receptors causes lung cell apoptosis and emphysema, *J Clin Invest*, 106, 1311-9. doi: 10.1172/jci10259.

[136] Zhao, H., Eguchi, S., Alam, A., and Ma, D. (2017). The role of nuclear factor-erythroid 2 related factor 2 (Nrf-2) in the protection against lung injury, *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 312, L155-L162. doi: 10.1152/ajplung.00449.2016.

[137] Sandberg, M., Patil, J., D'Angelo, B., Weber, S.G., and Mallard, C. (2014). NRF2-regulation in brain health and disease: Implication of cerebral inflammation, *Neuropharmacology*, 79, 298-306. doi: 10.1016/j.neuropharm.2013.11.004.

[138] Hseu, Y.-C., Chou, C.-W., Kumar, K.S., Fu, K.-T., Wang, H.-M., Hsu, L.-S., Kuo, Y.-H., Wu, C.-R., Chen, S.-C., and Yang, H.-L. (2012). Ellagic acid protects human keratinocyte (HaCaT) cells against UVA-induced oxidative stress and apoptosis through the upregulation of the HO-1 and Nrf-2 antioxidant genes, *Food and Chemical Toxicology*, 50, 1245-1255. doi: 10.1016/j.fct.2012.02.020.

[139] Rahman, I., Biswas, S.K., and Kode, A. (2006). Oxidant and antioxidant balance in the airways and airway diseases, *European Journal of Pharmacology*, 533, 222-239. doi: 10.1016/j.ejphar.2005.12.087.

[140] Surh, Y.-J., Kundu, J.K., and Na, H.-K. (2008). Nrf2 as a Master Redox Switch in Turning on the Cellular Signaling Involved in the Induction of Cytoprotective Genes by Some Chemopreventive Phytochemicals, *Planta Med*, 74, 1526-1539. doi: 10.1055/s-0028-1088302.

[141] Christofidou-Solomidou, M. and Muzykantov, V.R. (2006). Antioxidant Strategies in Respiratory Medicine, *Treatments in Respiratory Medicine*, 5, 47-78. doi: 10.2165/00151829-200605010-00004.

[142] Simon, R.H., DeHart, P.D., and Nadeau, D.M. (1989). Resistance of rat pulmonary alveolar epithelial cells to neutrophil- and oxidant-induced injury, *Am J Respir Cell Mol Biol*, 1, 221-9. doi: 10.1165/ajrcmb/1.3.221.

[143] Betsuyaku, T., Fuke, S., Inomata, T., Kaga, K., Morikawa, T., Odajima, N., Adair-Kirk, T., and Nishimura, M. (2013). Bronchiolar epithelial catalase is diminished in smokers with mild COPD, *European Respiratory Journal*, 42, 42-53. doi: 10.1183/09031936.00058912.

[144] Beane, J., Sebastiani, P., Liu, G., Brody, J.S., Lenburg, M.E., and Spira, A. (2007). Reversible and permanent effects of tobacco smoke exposure on airway epithelial gene expression, *Genome Biology*, 8, R201. doi: 10.1186/gb-2007-8-9-r201.

[145] Lomaestro, B.M. and Malone, M. (1995). Glutathione in Health and Disease: Pharmacotherapeutic Issues, *Annals of Pharmacotherapy*, 29, 1263-1273. doi: 10.1177/106002809502901213.

[146] Fang, Y.-Z., Yang, S., and Wu, G. (2002). Free radicals, antioxidants, and nutrition, *Nutrition*, 18, 872-879. doi: https://doi.org/10.1016/S0899-9007(02)00916-4.

[147] Lobo, V., Patil, A., Phatak, A., and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacognosy reviews*, 4, 118-126. doi: 10.4103/0973-7847.70902.

[148] Girish, C., Koner, B.C., Jayanthi, S., Ramachandra Rao, K., Rajesh, B., and Pradhan, S.C. (2009). Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice, *Fundamental & Clinical Pharmacology*, 23, 735-745. doi: 10.1111/j.1472-8206.2009.00722.x.

[149] Kidd, P.M. (1997). Glutathione : systematic protectant against oxidative and free radical damage, *Altern Med Rev*, 2, 155-176.

[150] Kode, A., Rajendrasozhan, S., Caito, S., Yang, S.-R., Megson, I.L., and Rahman, I. (2008). Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells, *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 294, L478-L488. doi: 10.1152/ajplung.00361.2007.

[151] Gould, N.S., Min, E., Gauthier, S., Martin, R.J., and Day, B.J. (2011). Lung glutathione adaptive responses to cigarette smoke exposure, *Respiratory research*, 12, 133. doi: 10.1186/1465-9921-12-133.

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